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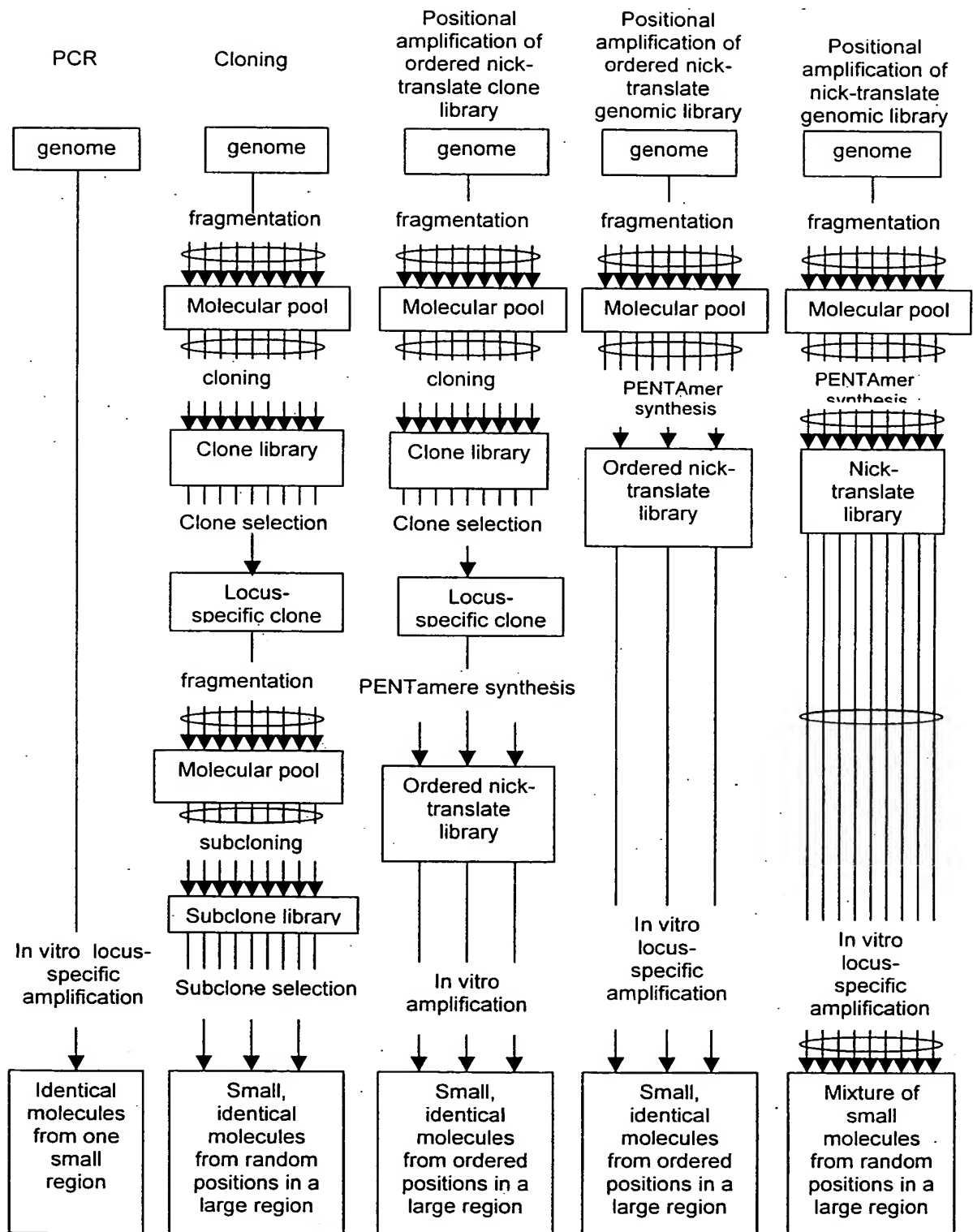


FIG. 1

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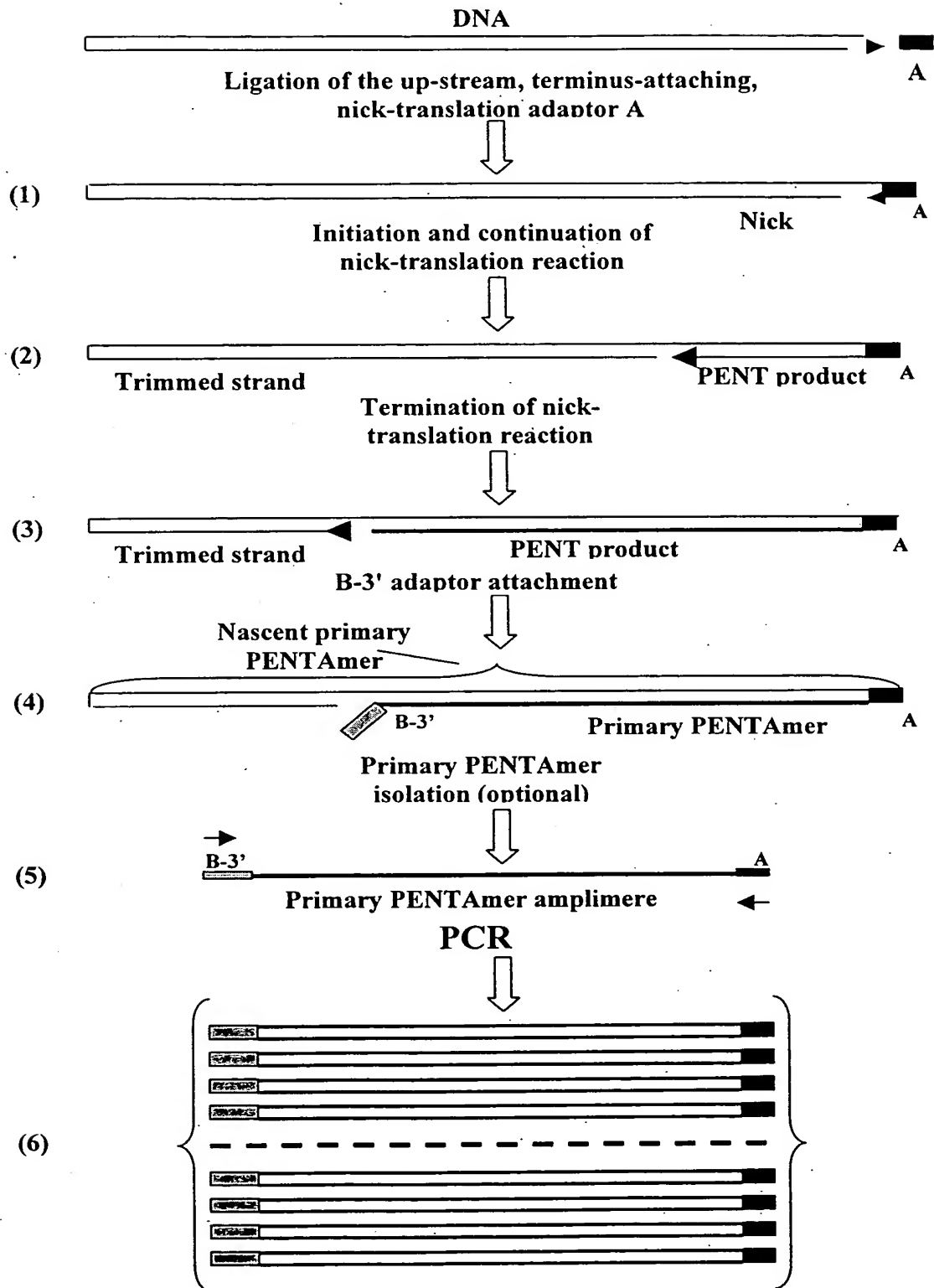


FIG. 2A

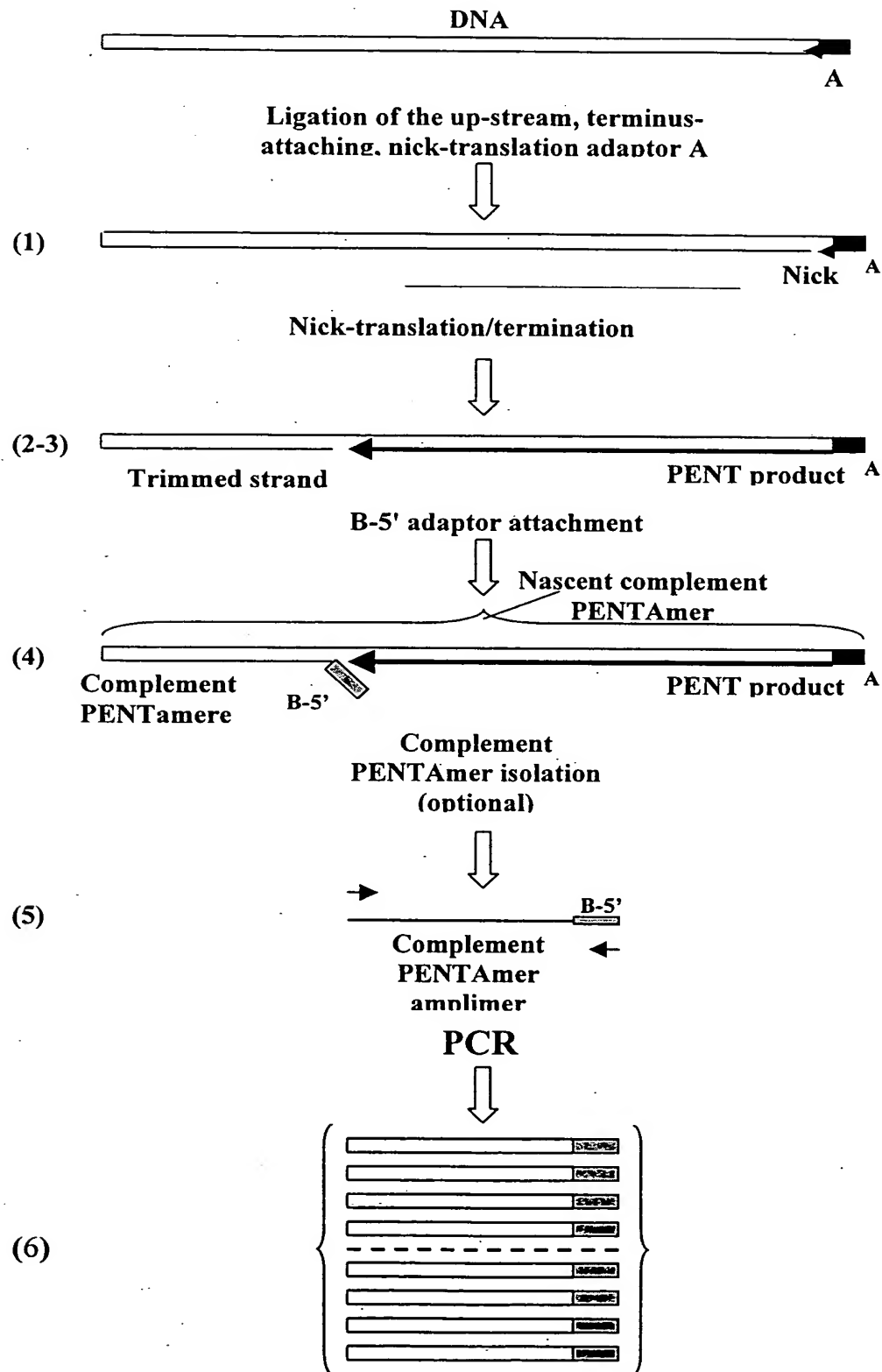


FIG. 2B



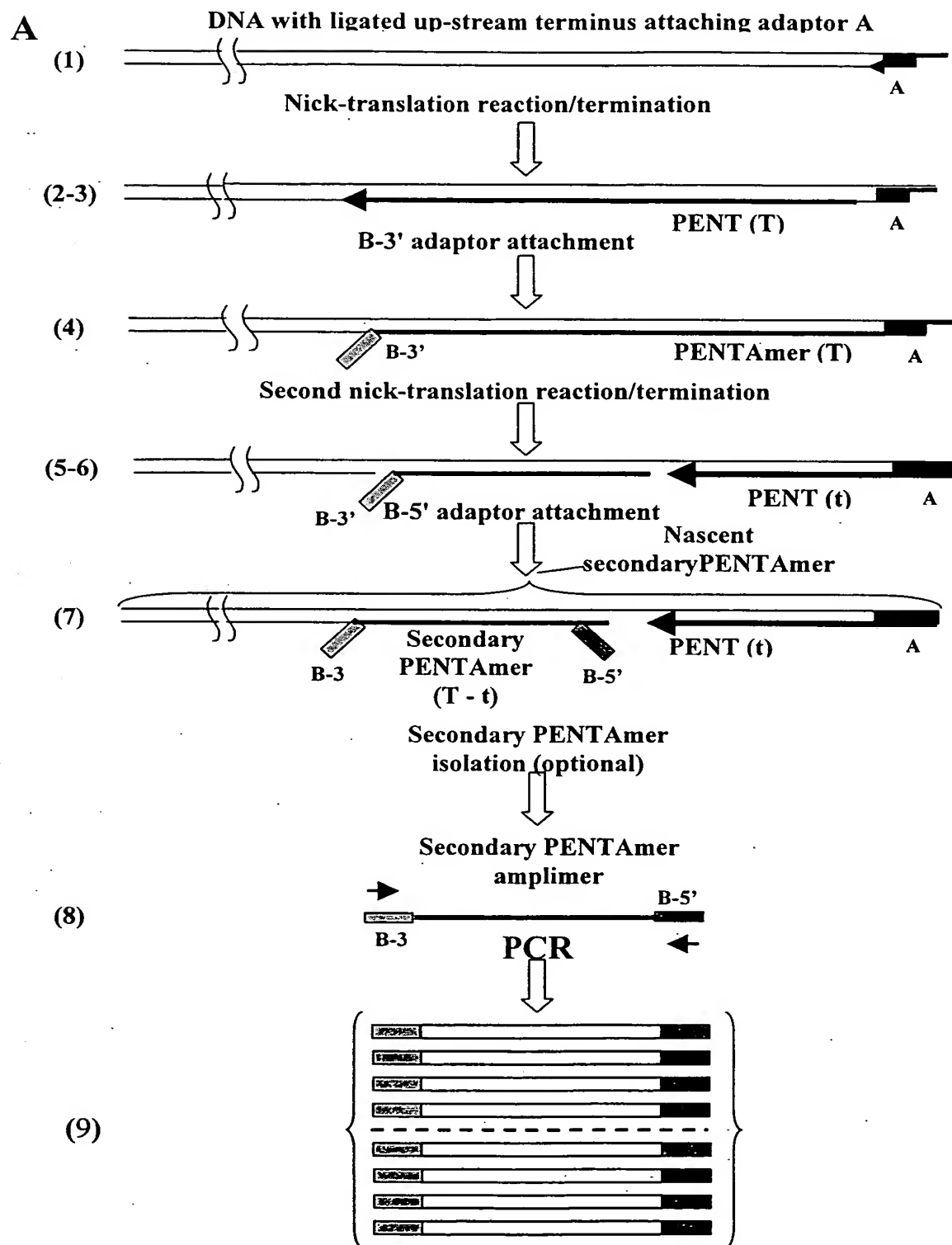


FIG. 3A

**B**

(1 - 3)

as in FIG. 3A

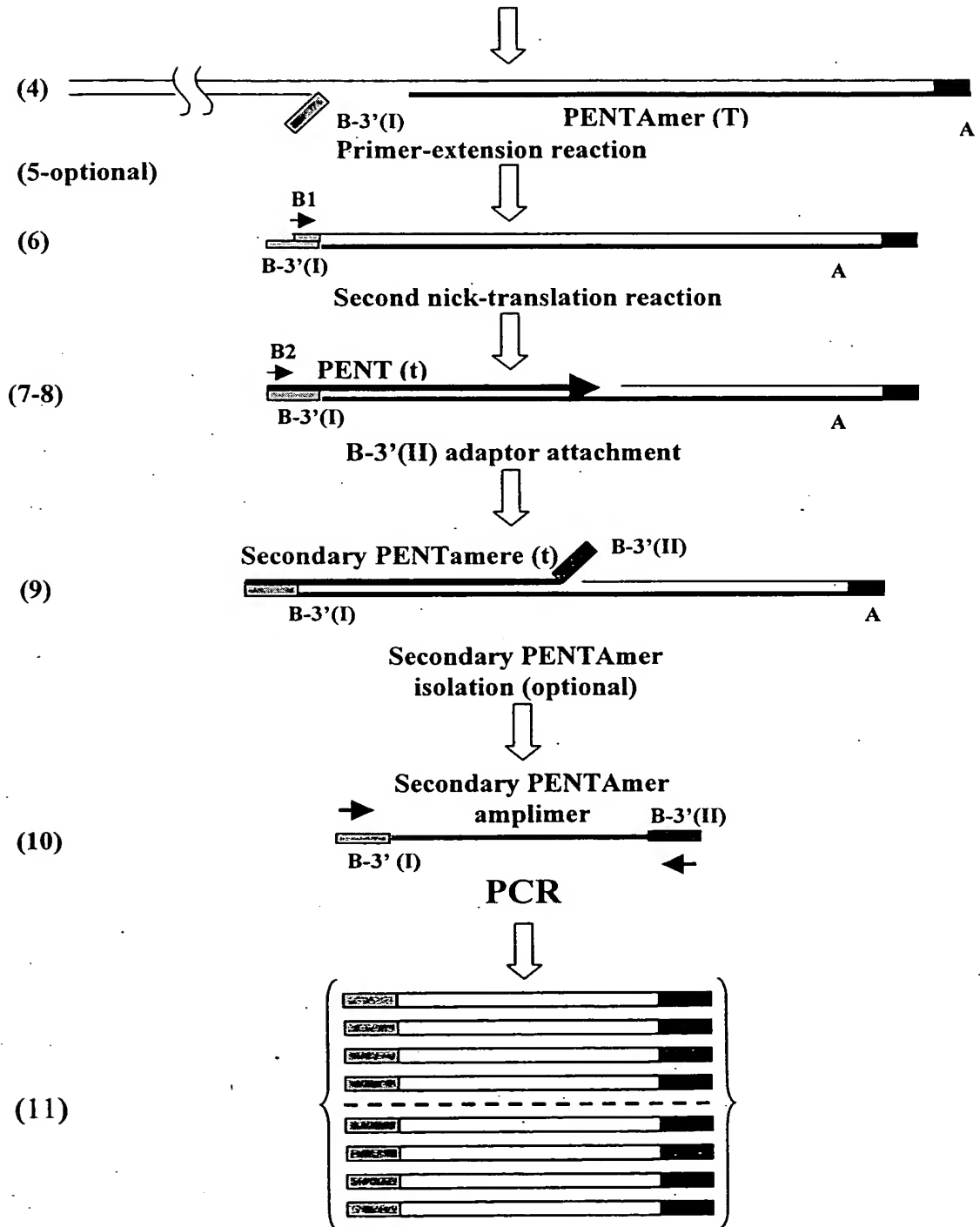


FIG. 3B

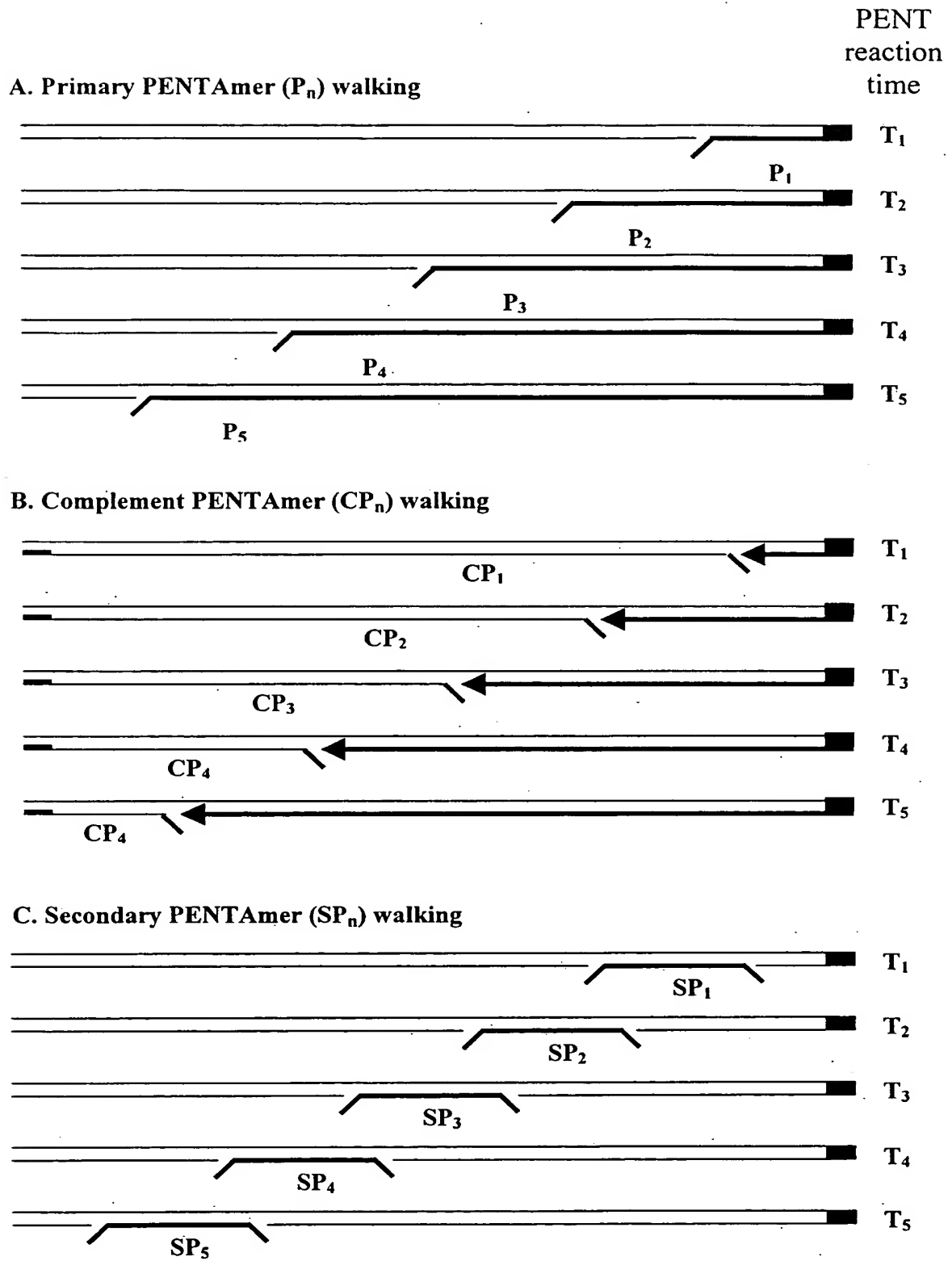


FIG. 4

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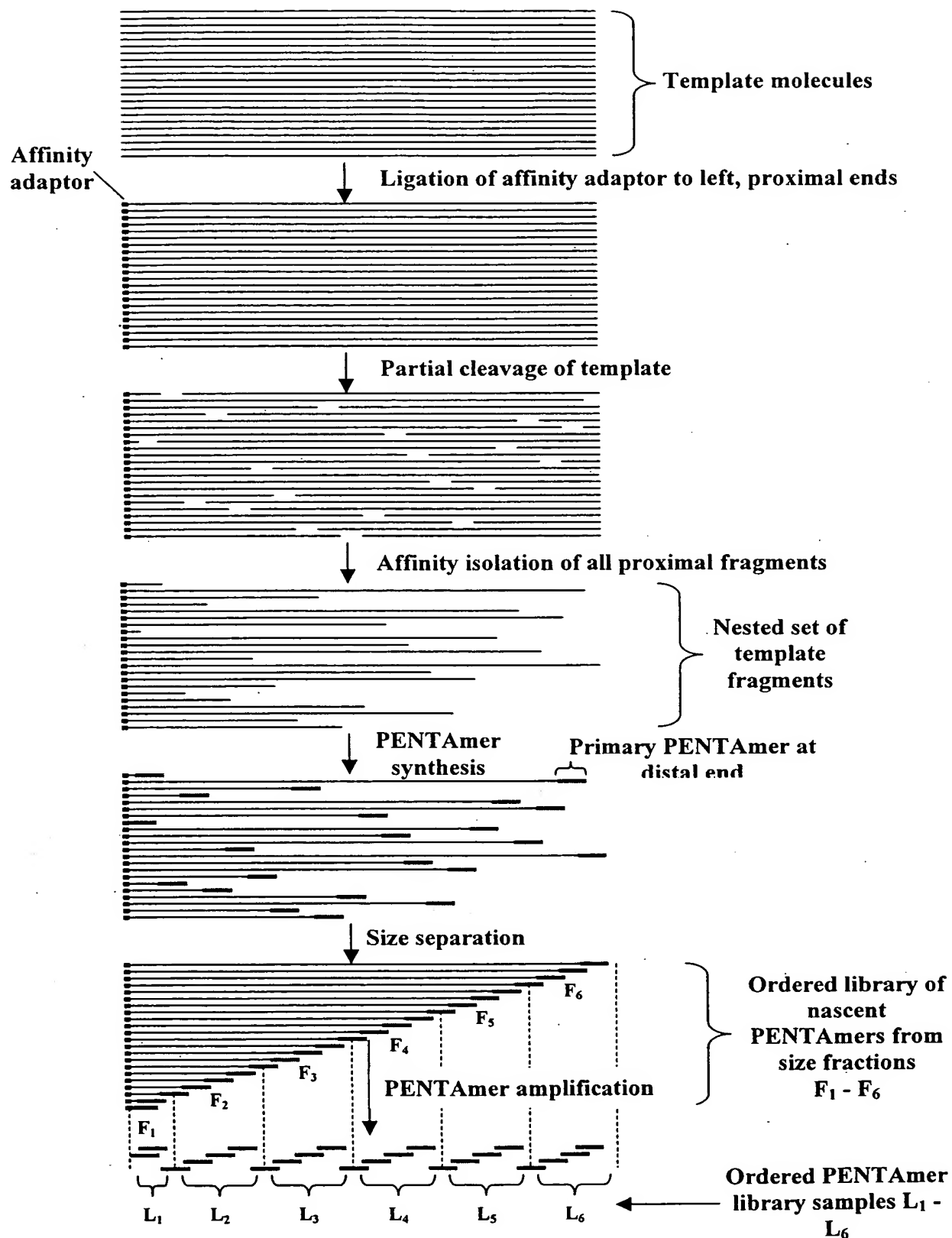


FIG. 5

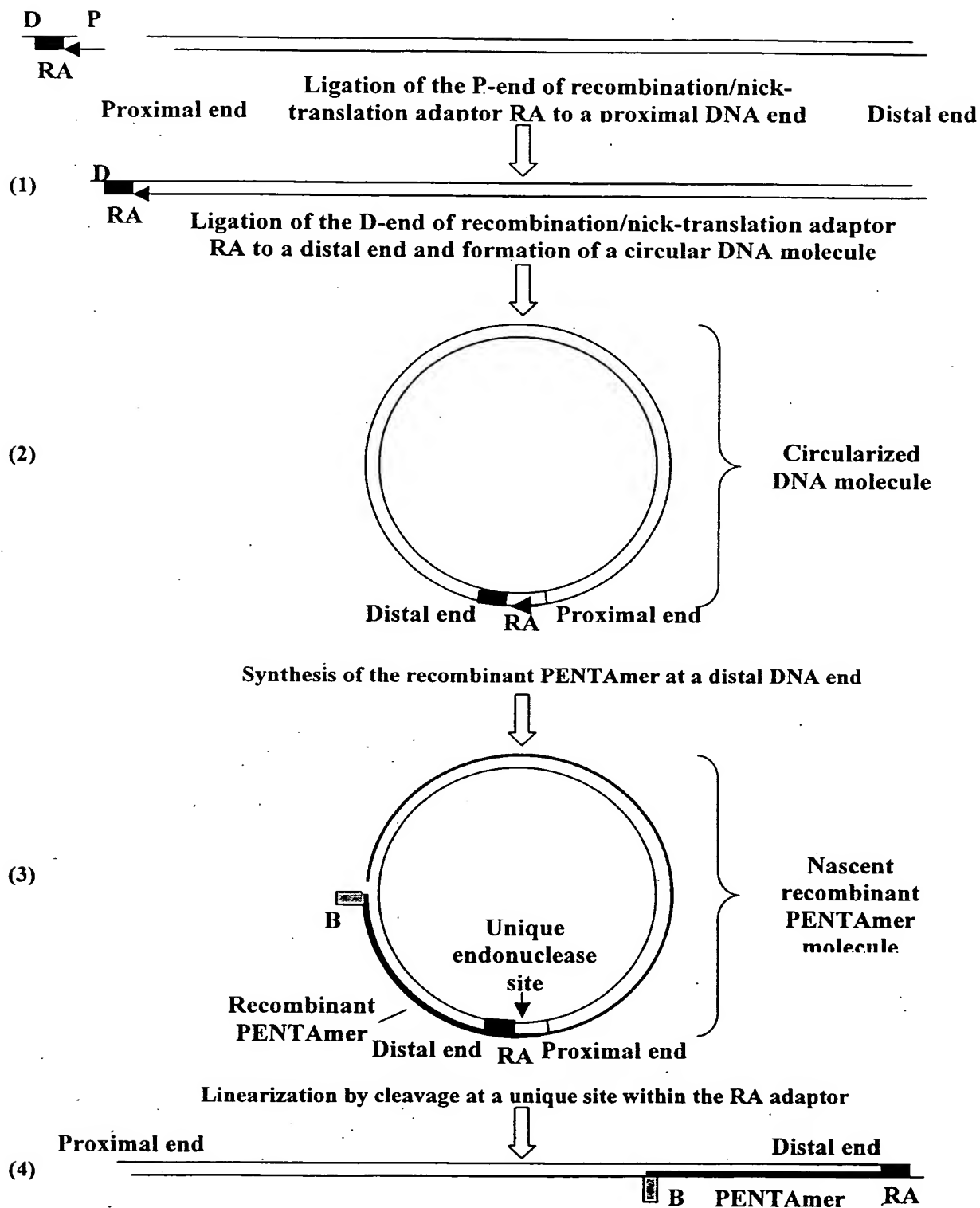


FIG. 6

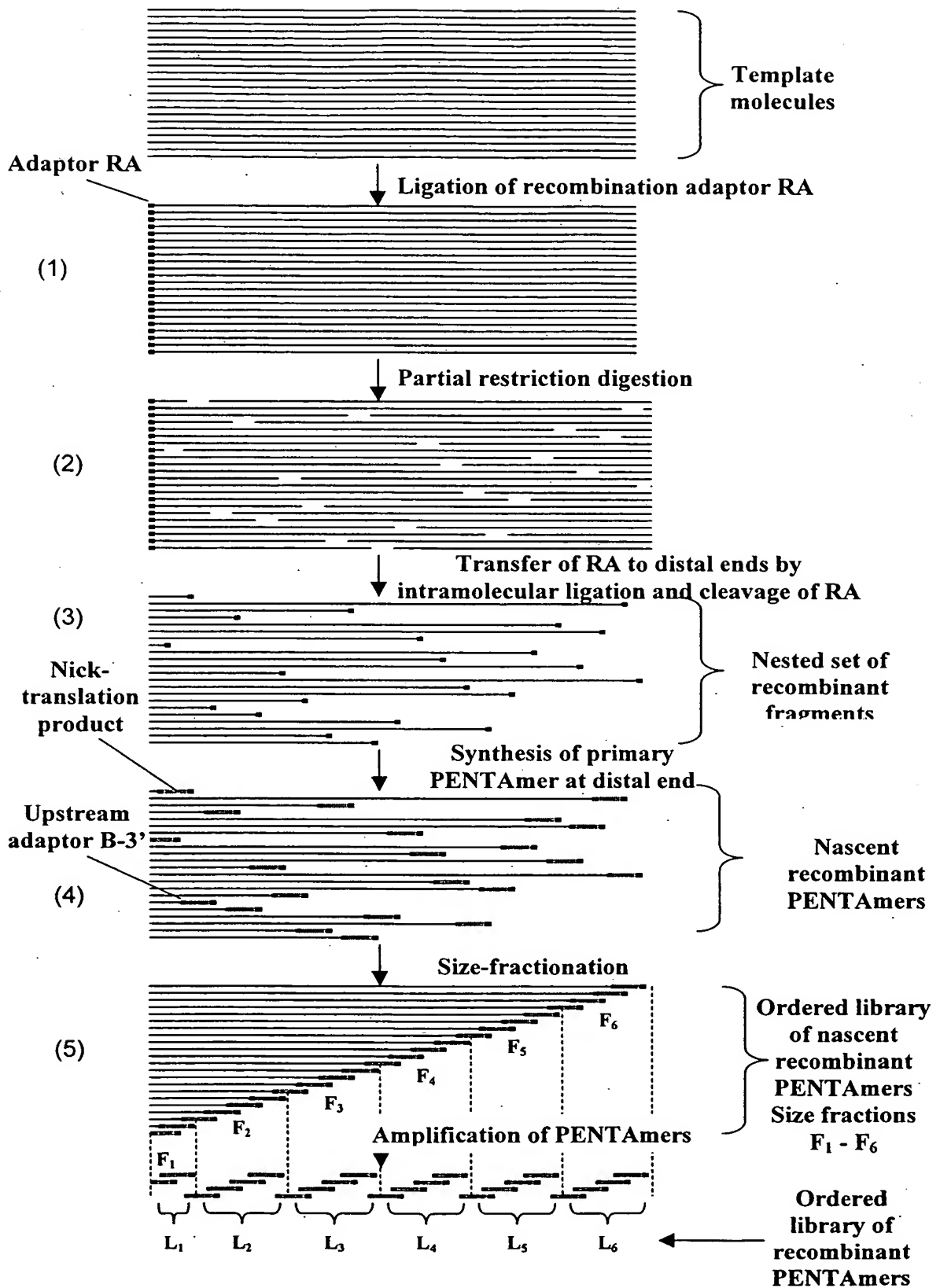


FIG. 7

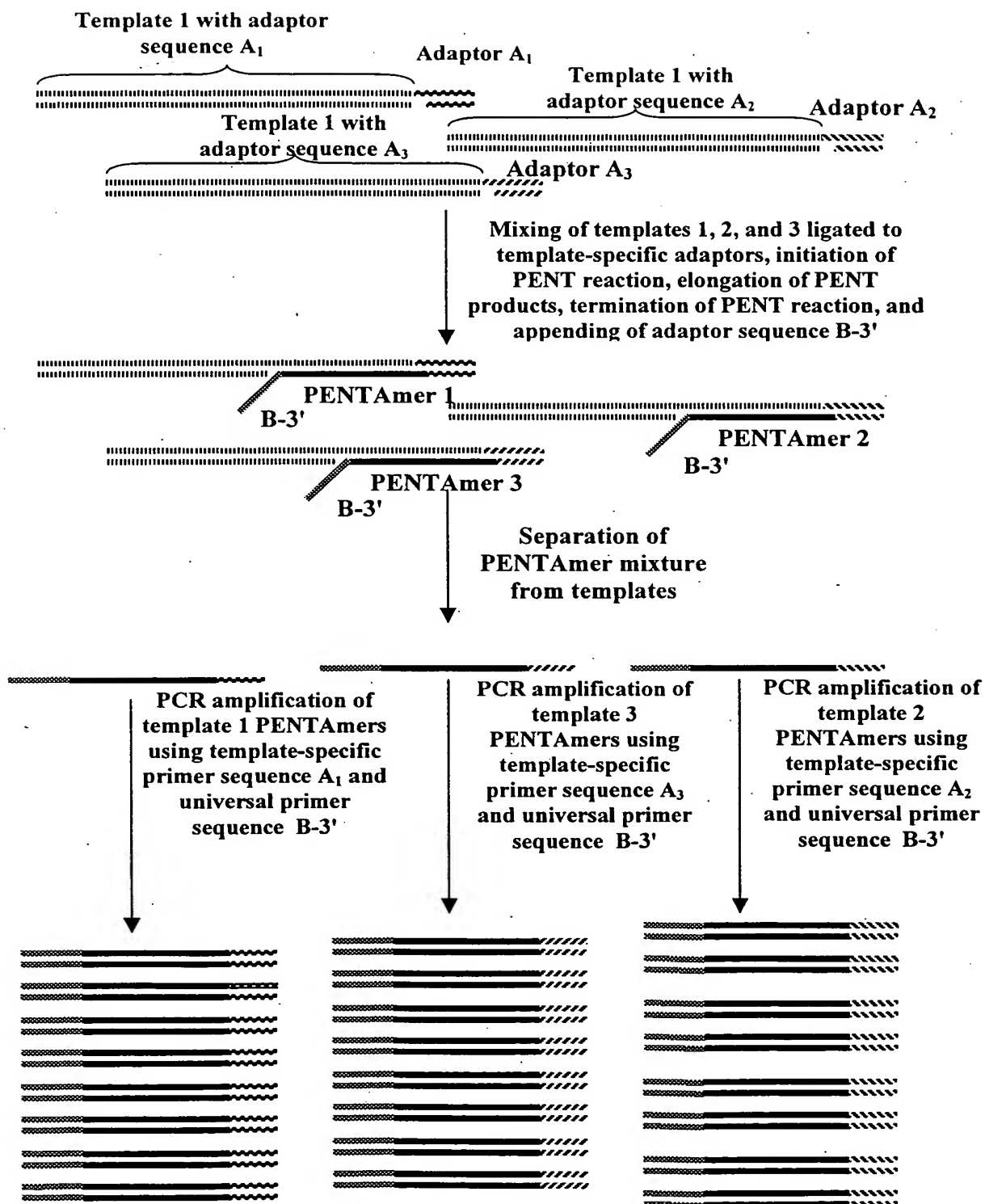


FIG. 8

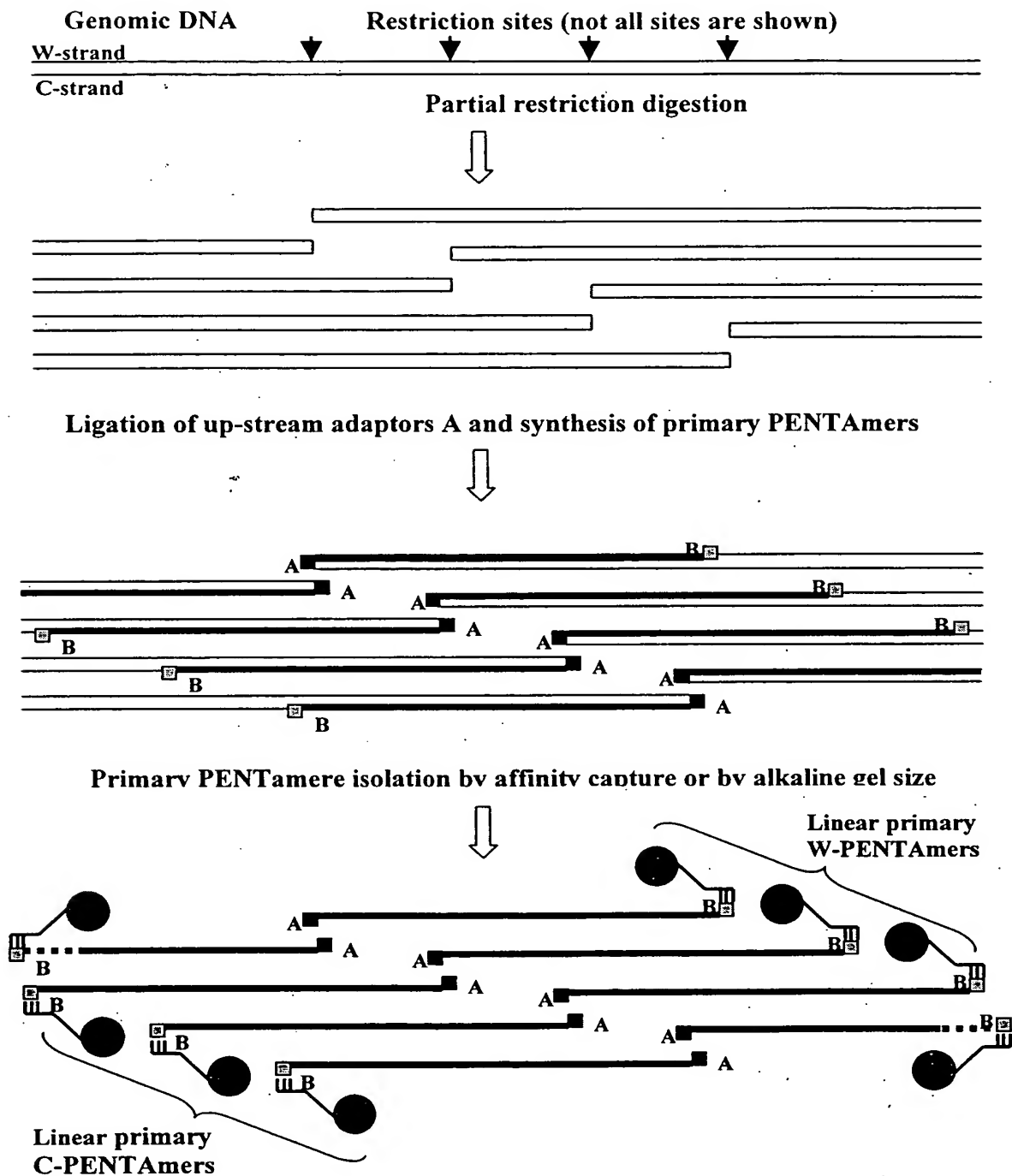


FIG. 9A



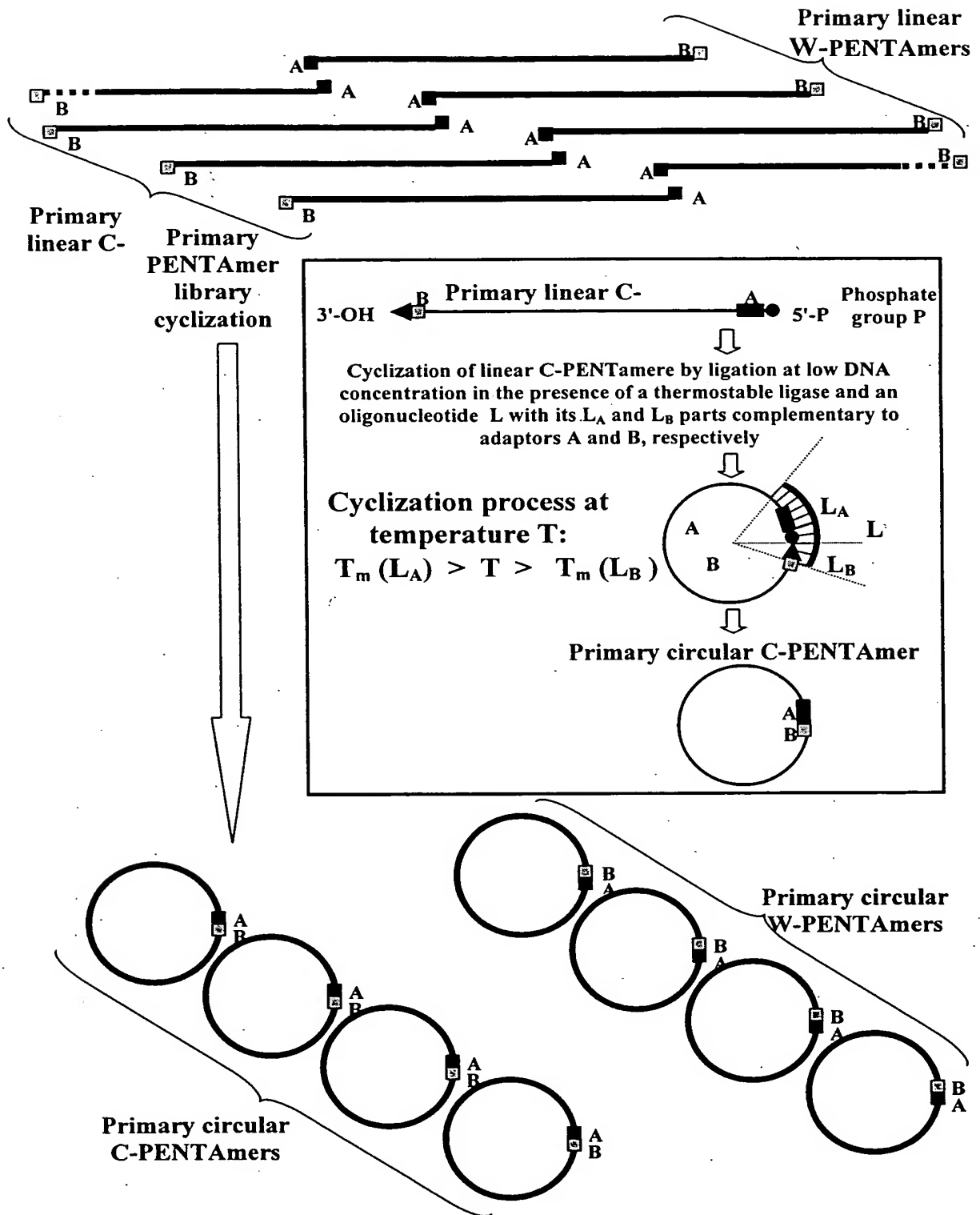


FIG. 9B

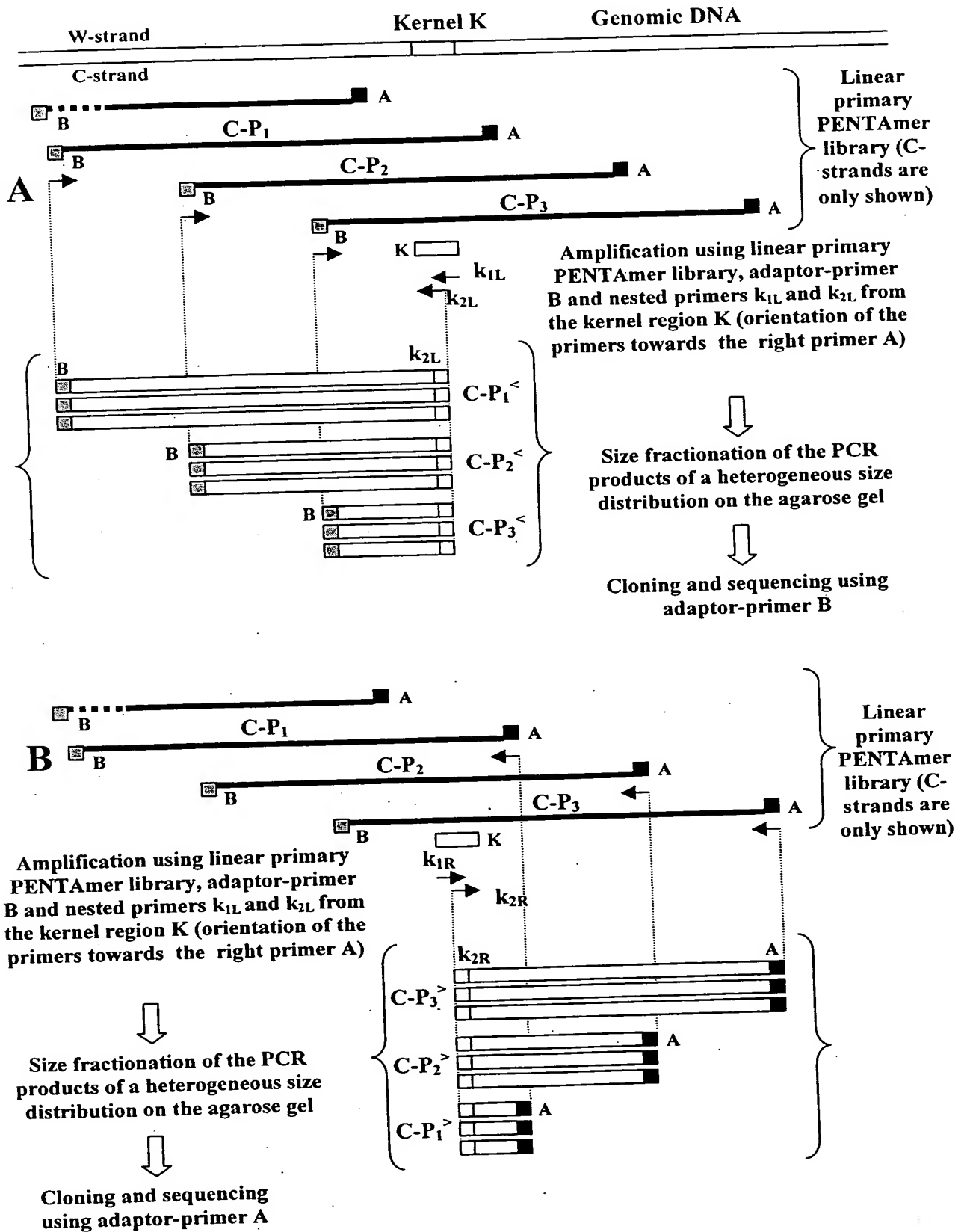


FIG. 10

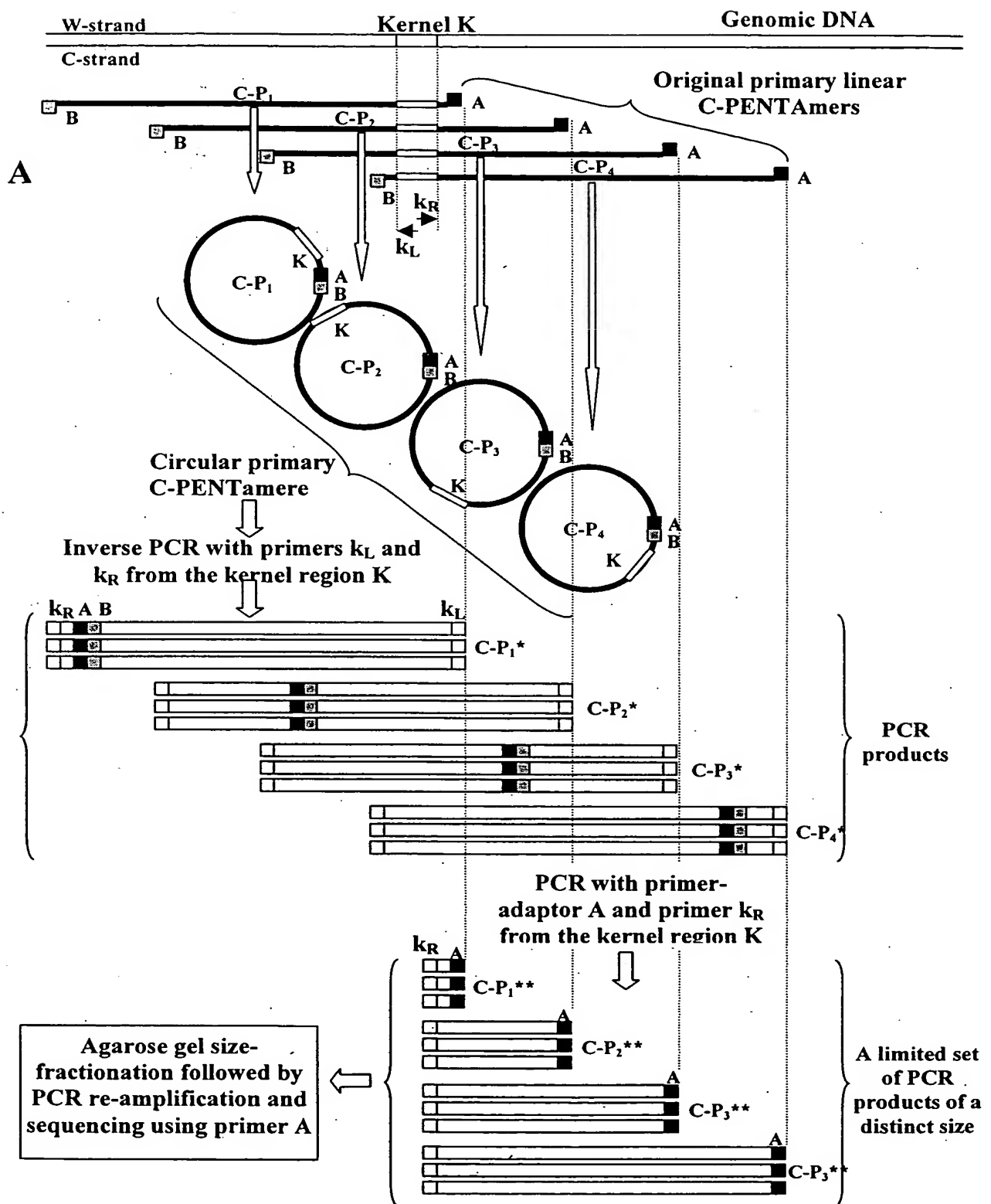


FIG. 11A

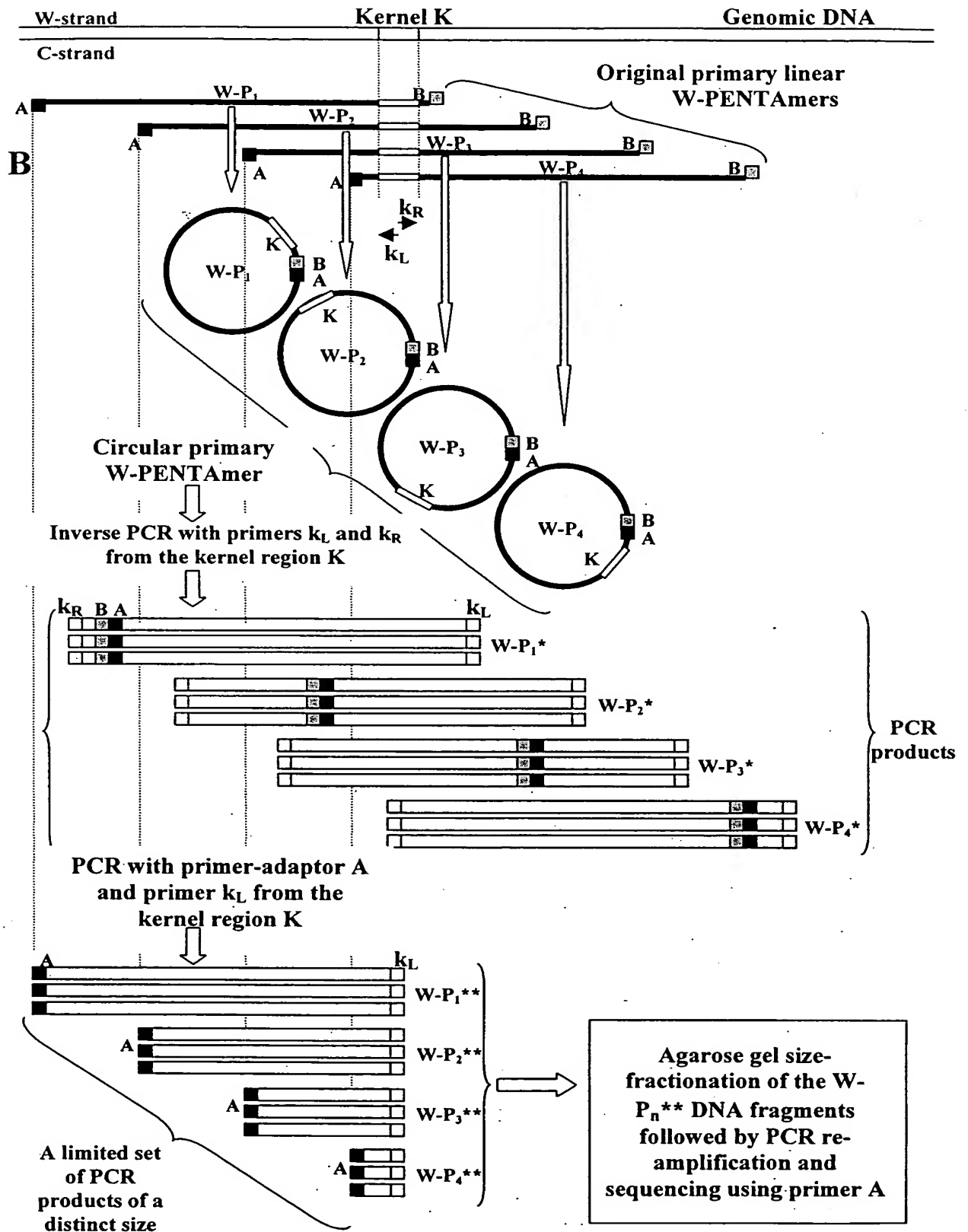


FIG. 11B

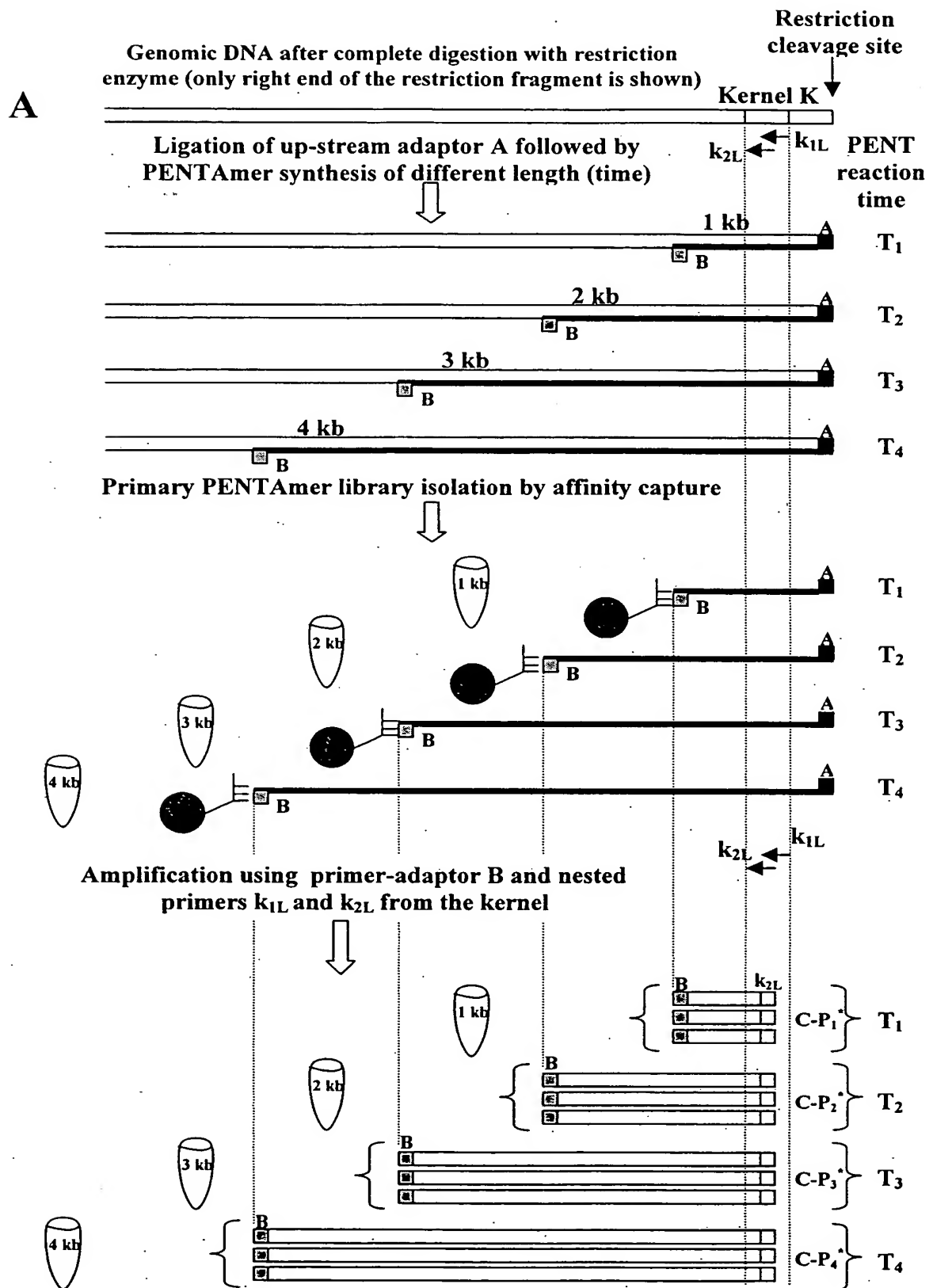
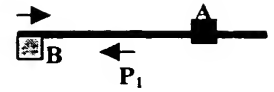
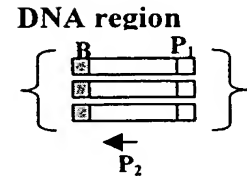


FIG. 12A

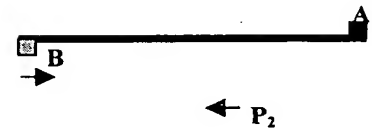
**B** Amplification using 1 kb library, primer-adaptor B and primer P<sub>1</sub> from the kernel region



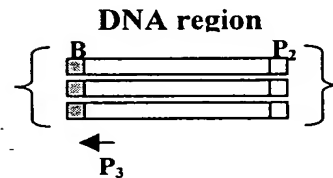
Cloning and sequencing PCR products followed by synthesis of the primer P<sub>2</sub> from the sequenced DNA region 1



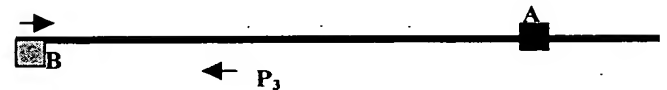
Amplification using 2 kb library, primer-adaptor B and primer P<sub>2</sub> from the sequenced region 1



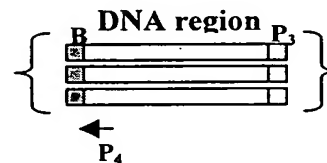
Cloning and sequencing PCR products followed by synthesis of the primer P<sub>3</sub> from the sequenced DNA region 2



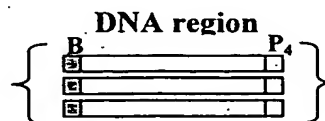
Amplification using 3 kb library, primer-adaptor B and primer P<sub>3</sub> from the sequenced region 2



Cloning and sequencing PCR products followed by synthesis of the primer P<sub>4</sub> from the sequenced DNA region 3



Amplification using 4 kb library, primer-adaptor B and primer P<sub>4</sub> from the sequenced region 3



Cloning and sequencing PCR products (region 4)

FIG. 12B

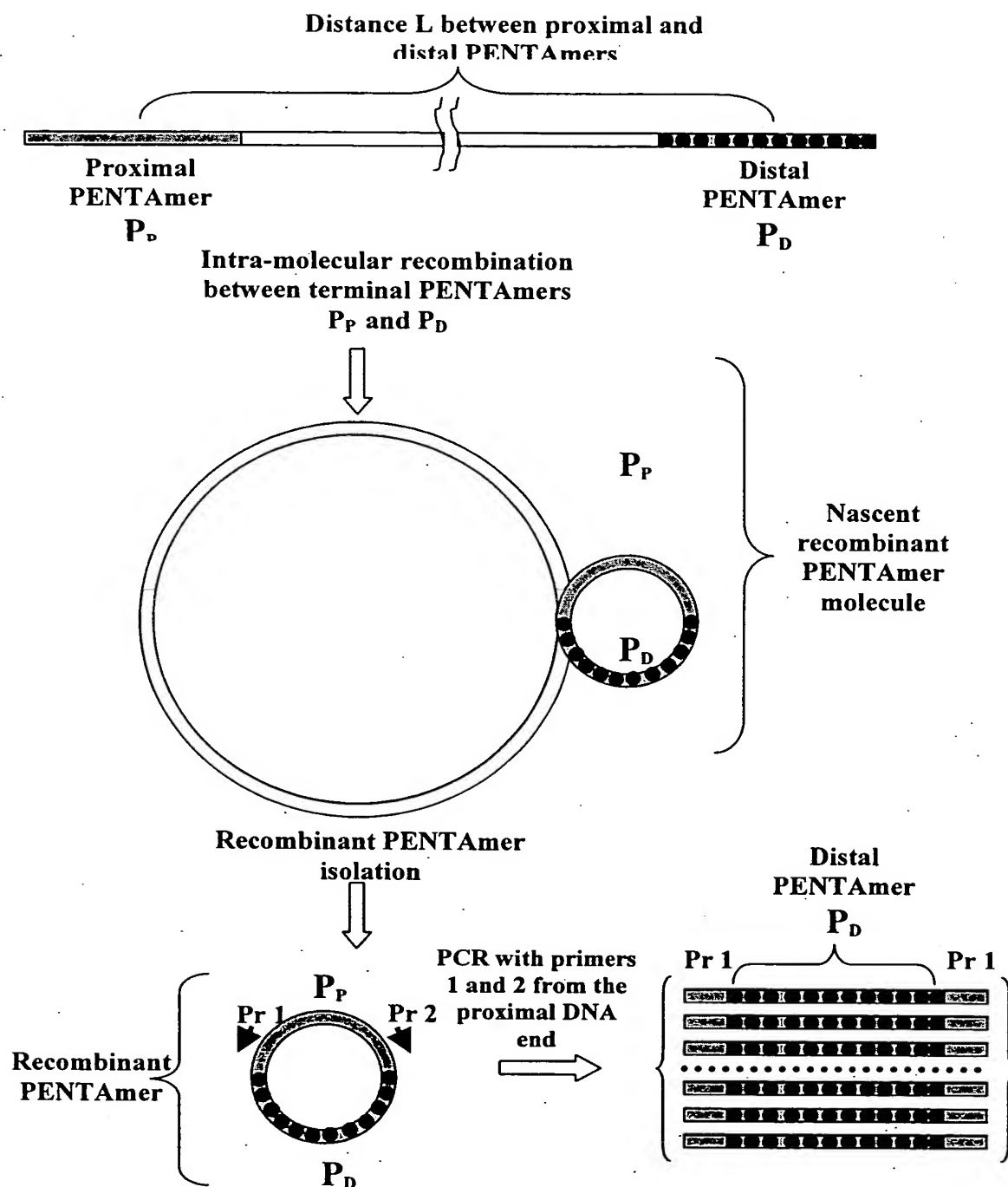
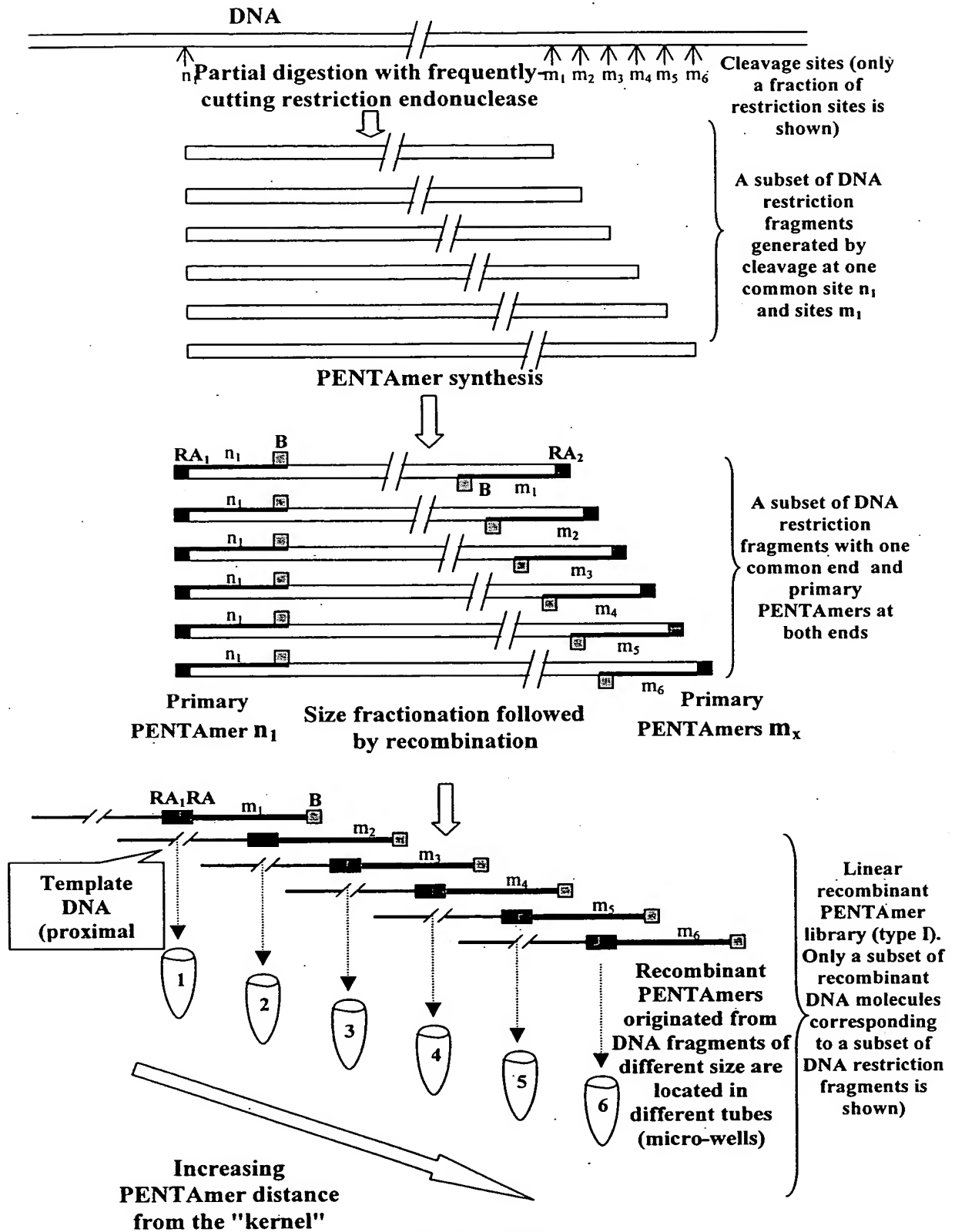


FIG. 13





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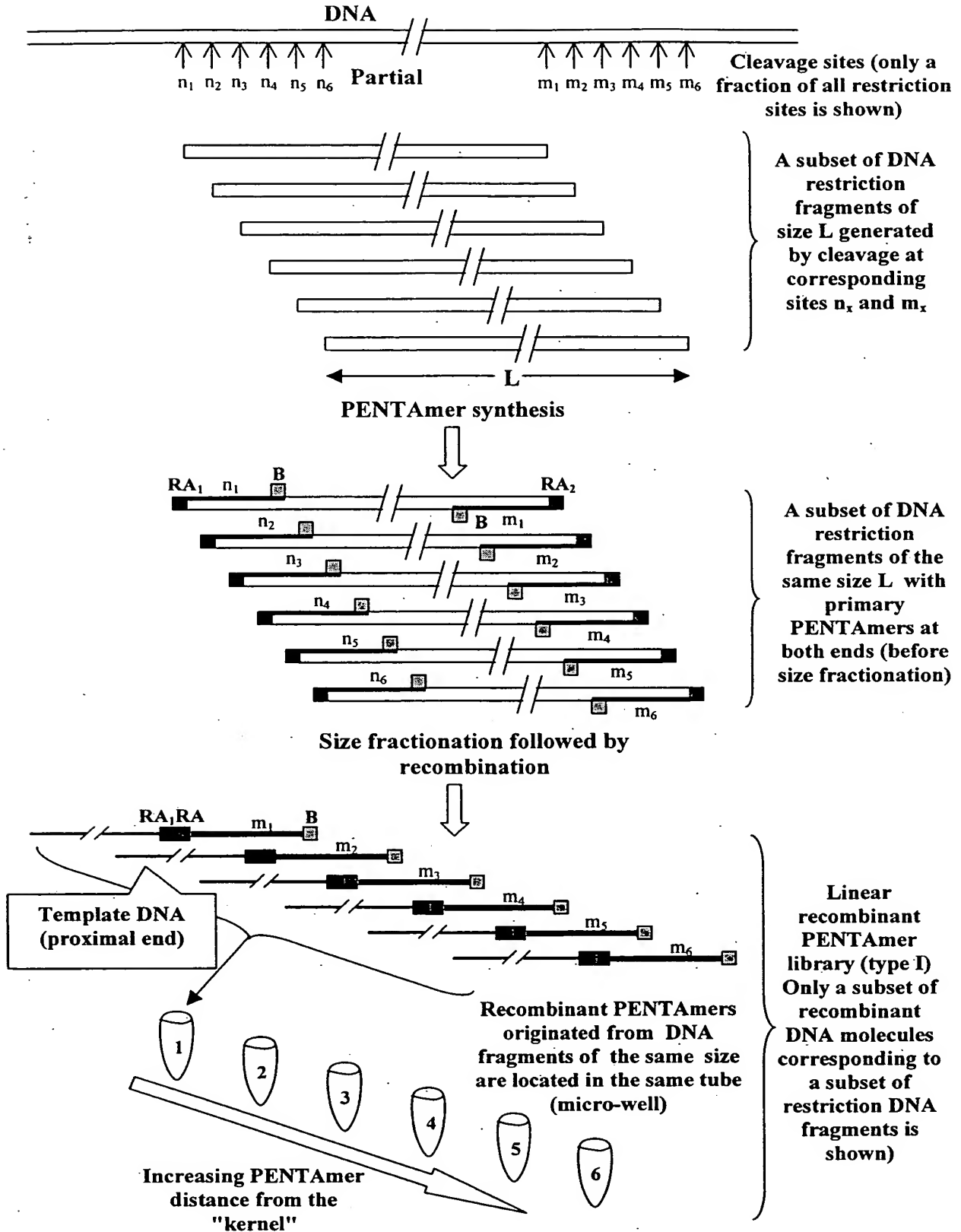


FIG. 14B

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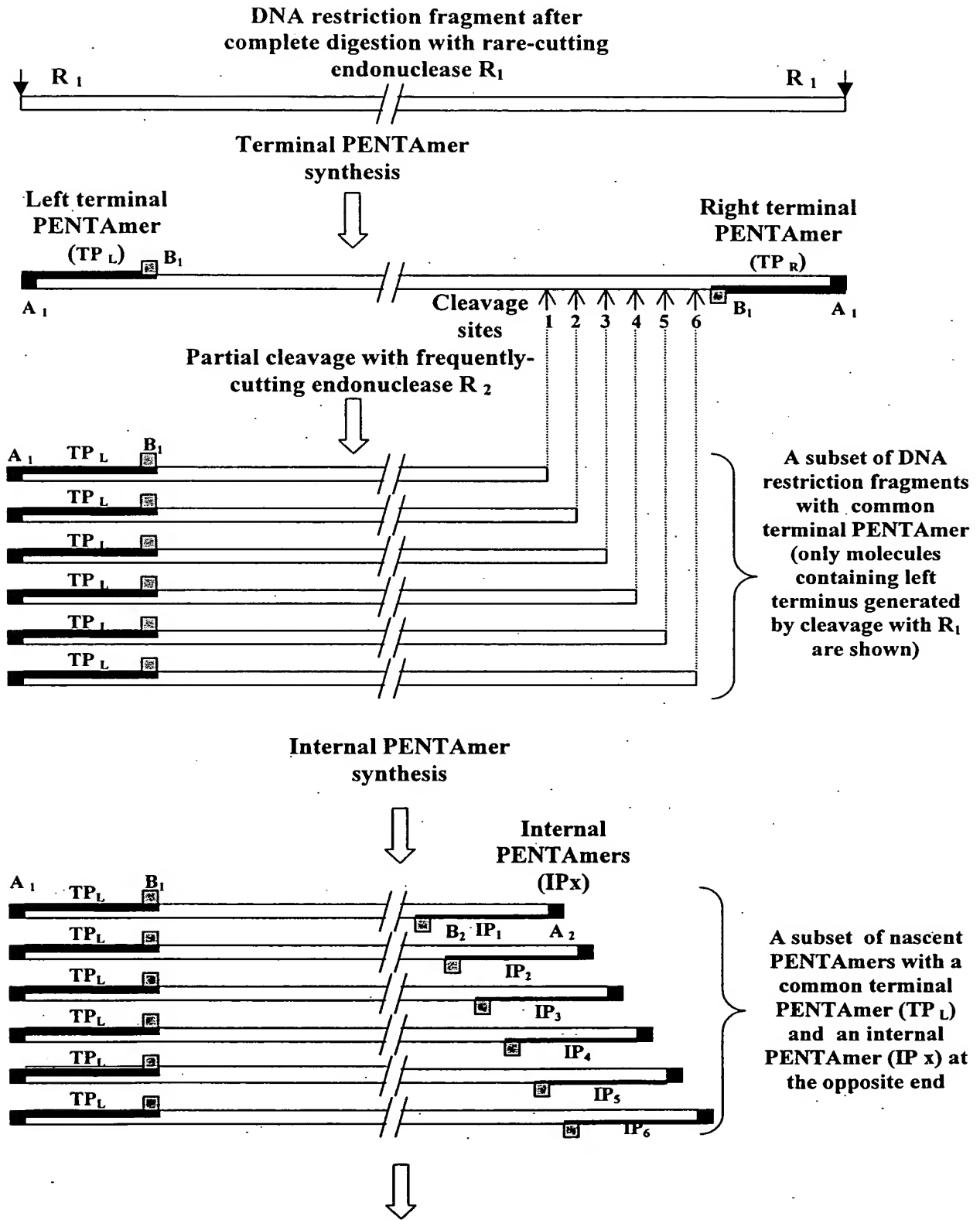


FIG. 15A

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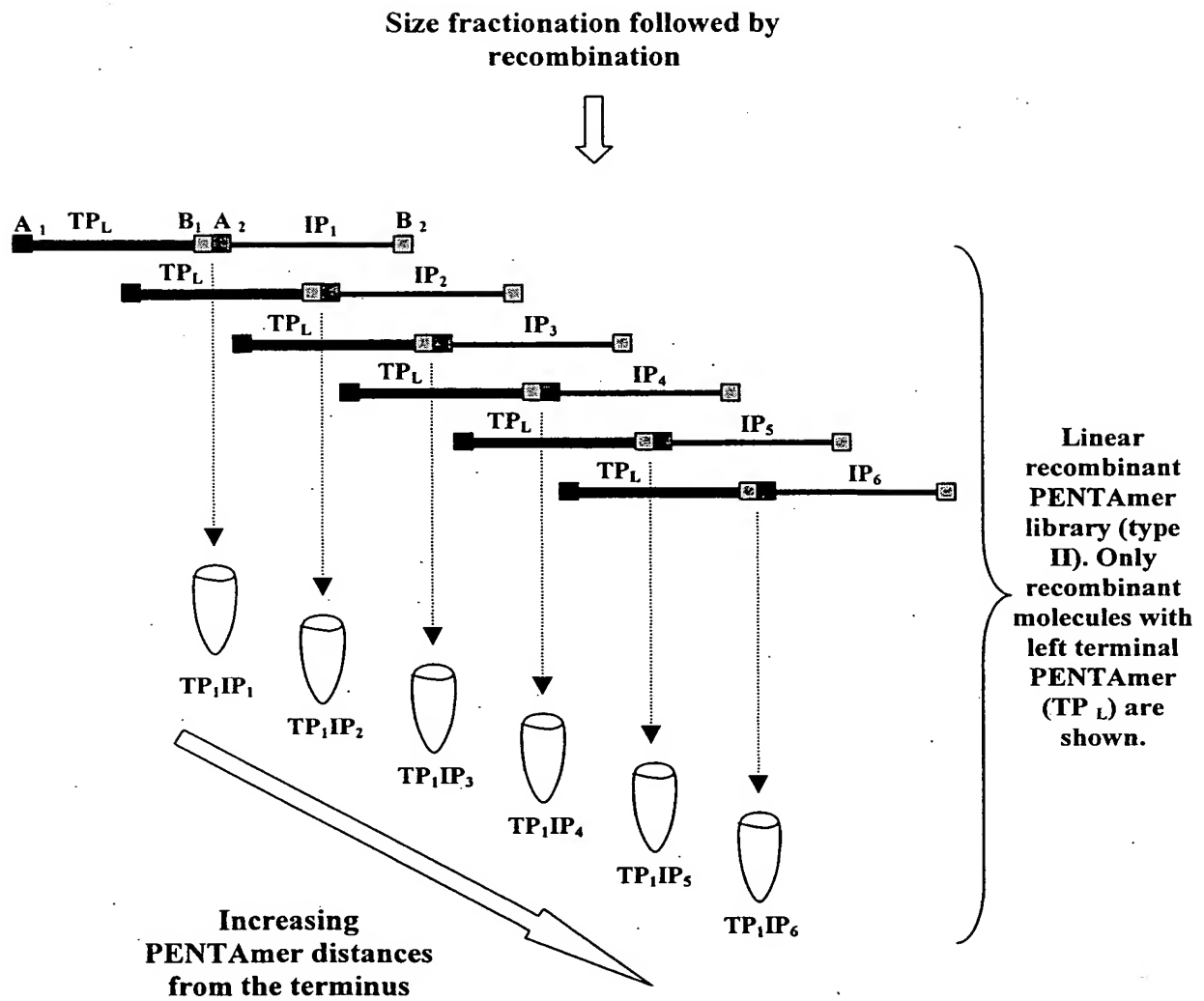


FIG. 15B

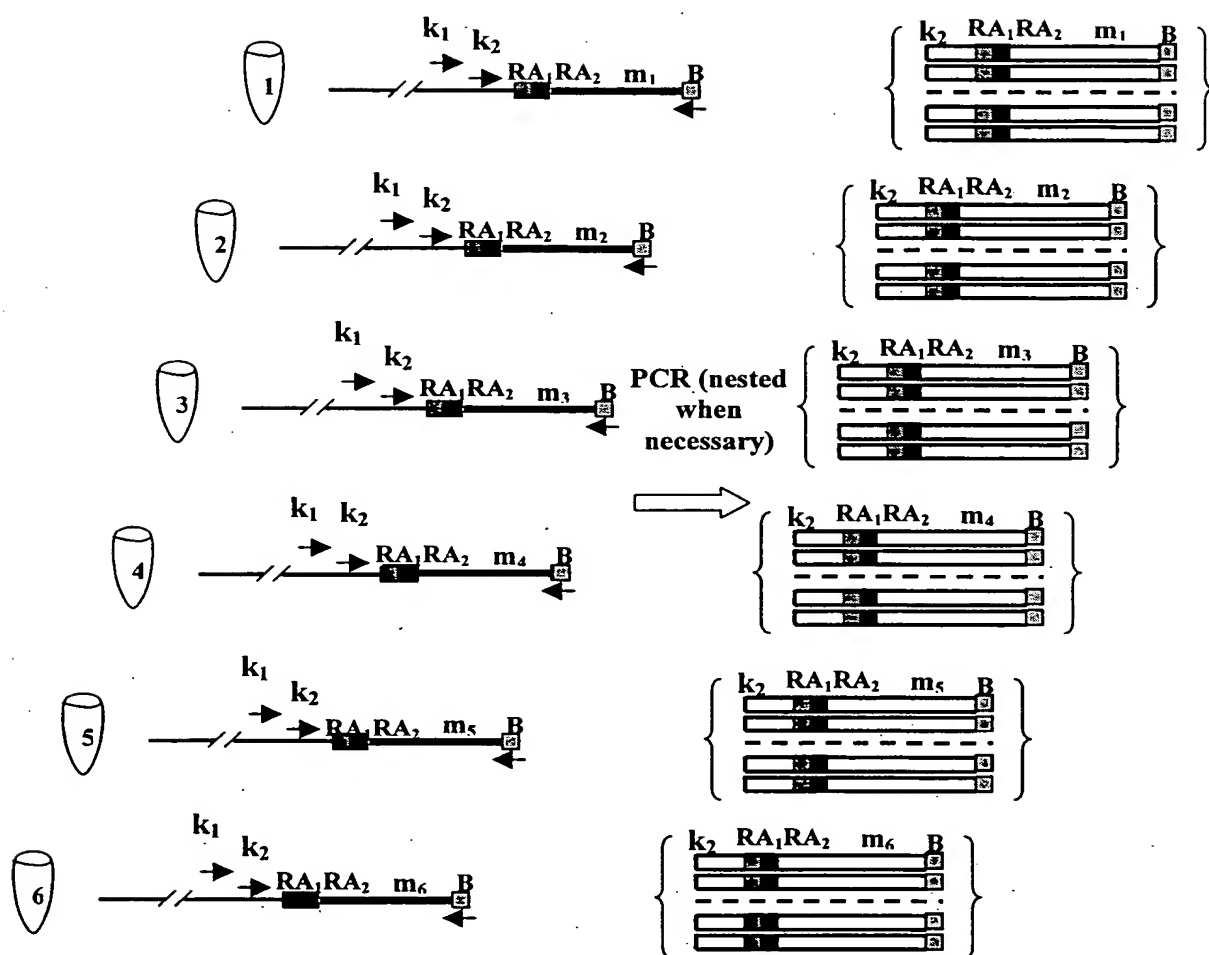
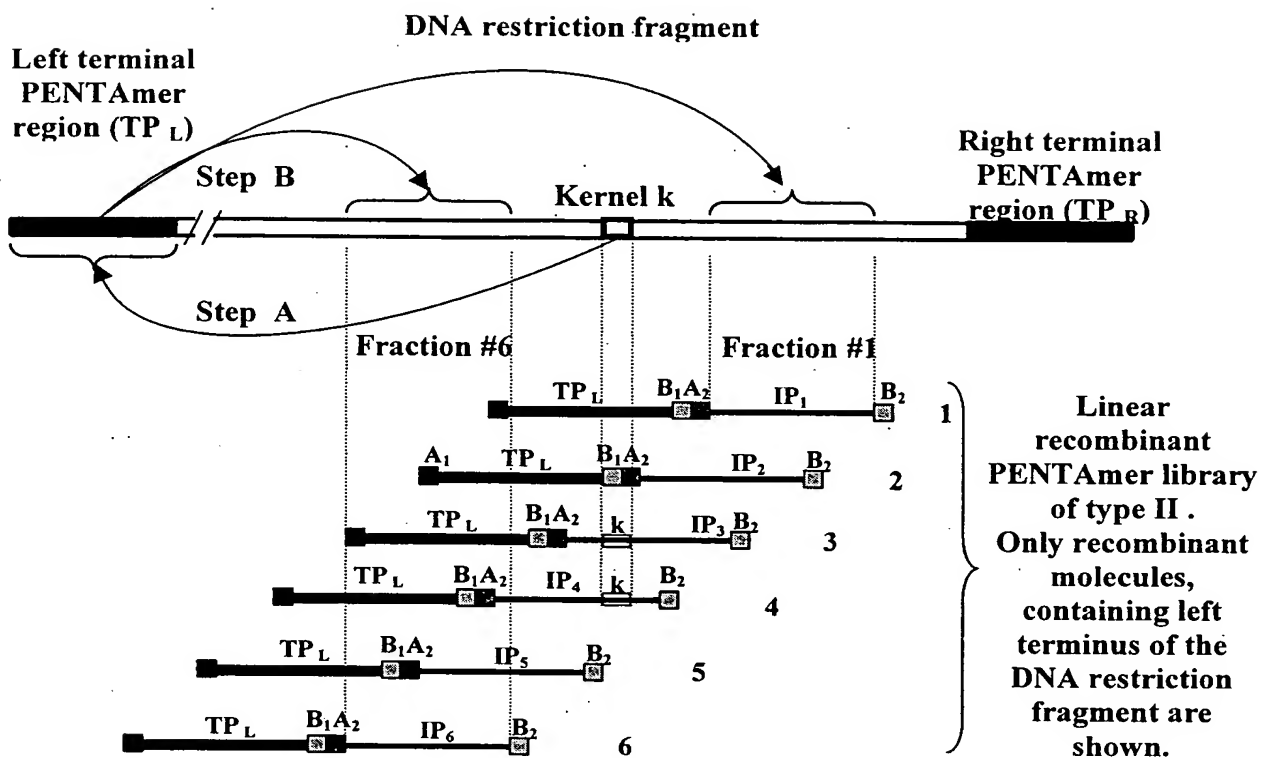


FIG. 16



**Step A. Amplification of terminal PENTAMers (TP) of a DNA restriction fragment using "One-tube" unordered library II, nested primers K<sub>1</sub> and K<sub>2</sub> from the internal "kernel" region k and adaptor-primer A<sub>1</sub> (only amplification of left terminal PENTamer is shown)**

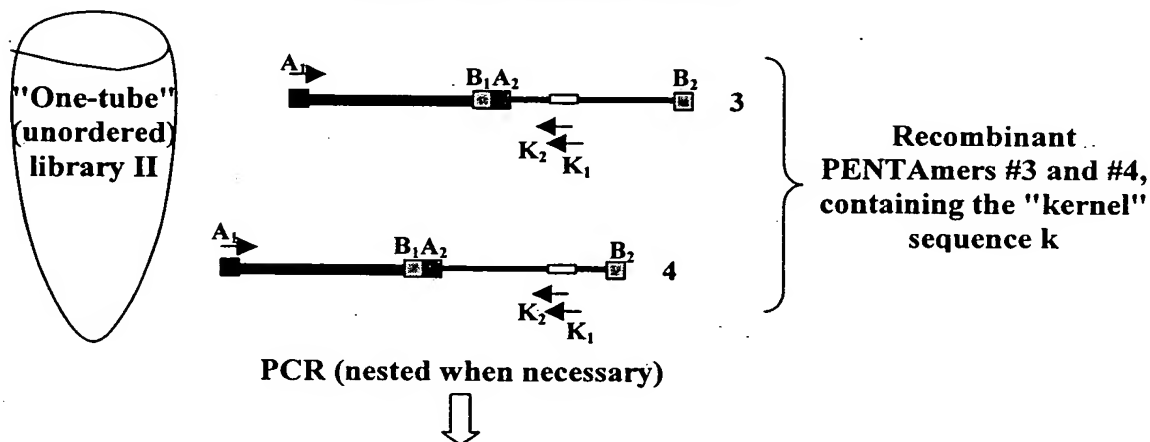
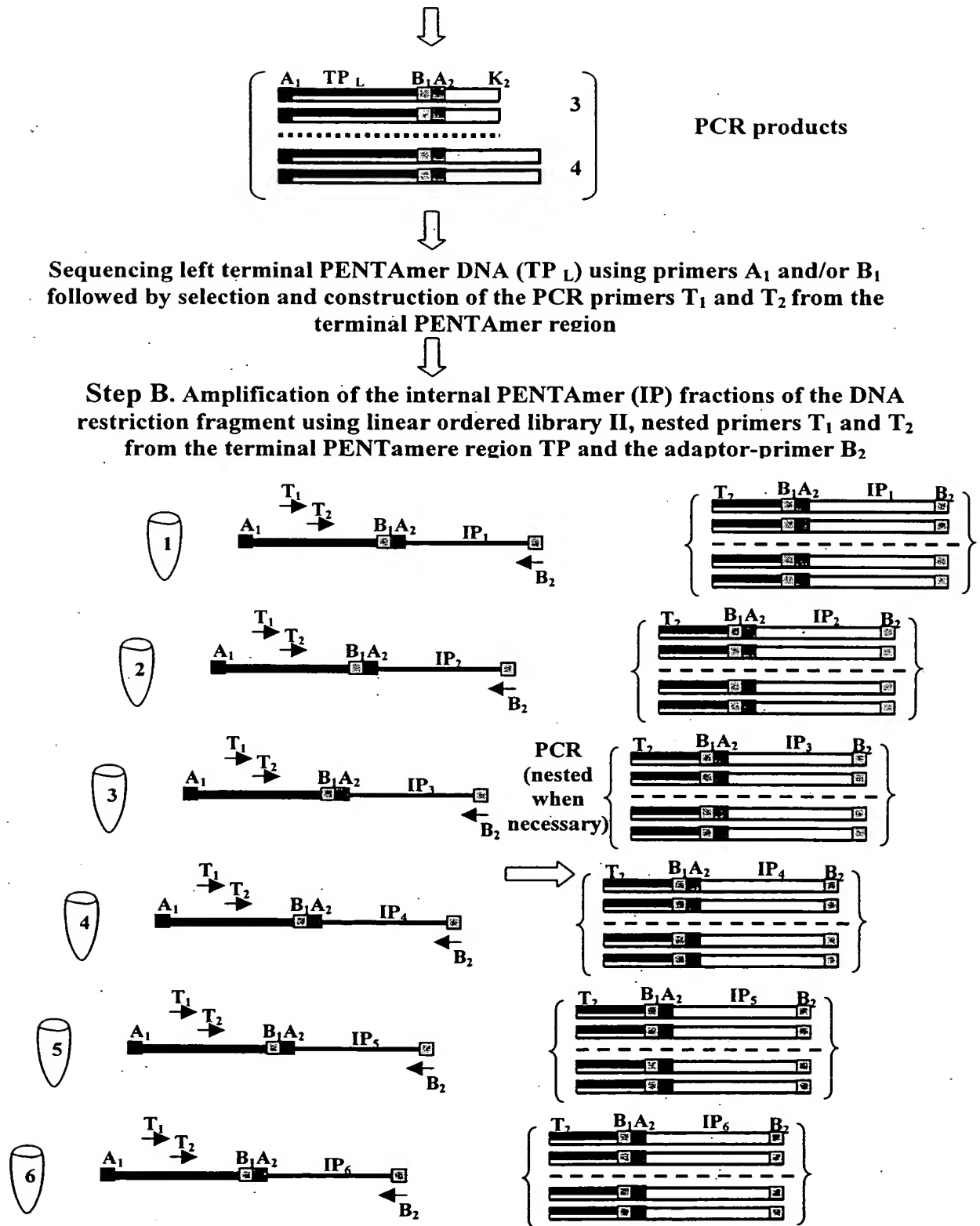
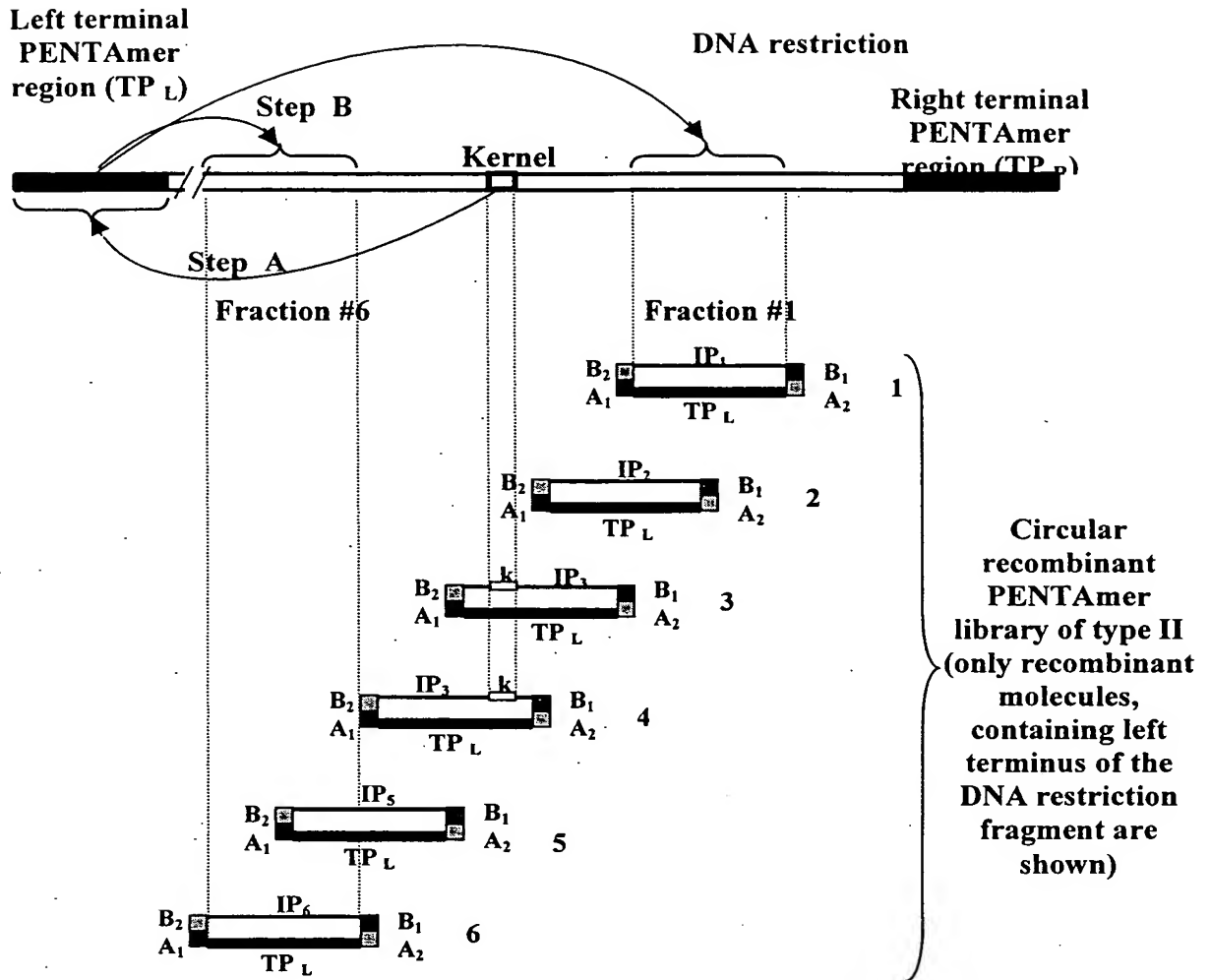


FIG. 17A

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**Step A. Amplification of terminal PENTAMers (TP) of a DNA restriction fragment using "One-tube" circular library II, inverse primers K<sub>1</sub> and K<sub>2</sub> from the internal "kernel" region k**

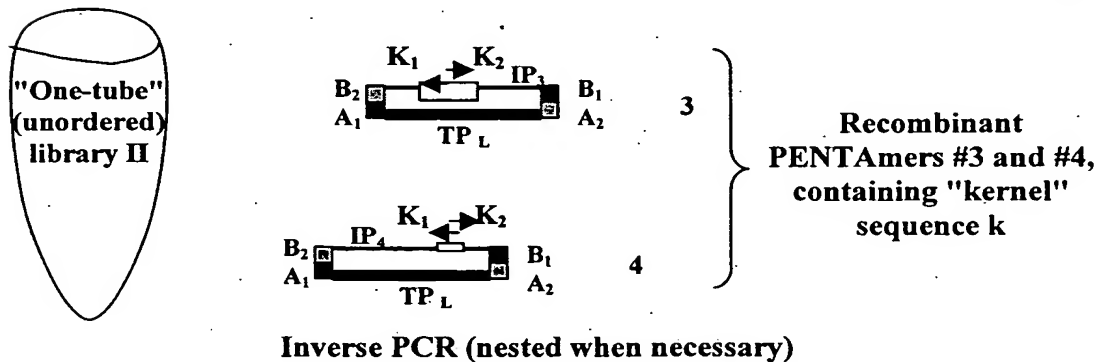
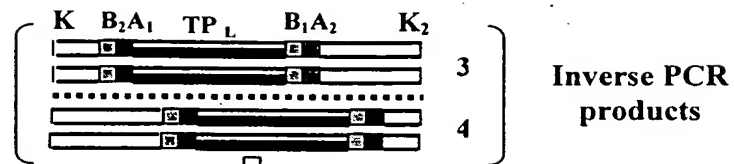


FIG. 17C



Sequencing a terminal PENTamer DNA (TP) using primers  $A_1$  and /or  $B_1$  followed by selection and construction of two inverse PCR primers  $T_1$  and  $T_2$

**Step B. Amplification of the internal PENTamer (IP) fractions of the DNA restriction fragment using inverse primers  $T_1$  and  $T_2$  from the terminal PENTamer region and ordered circular library II**

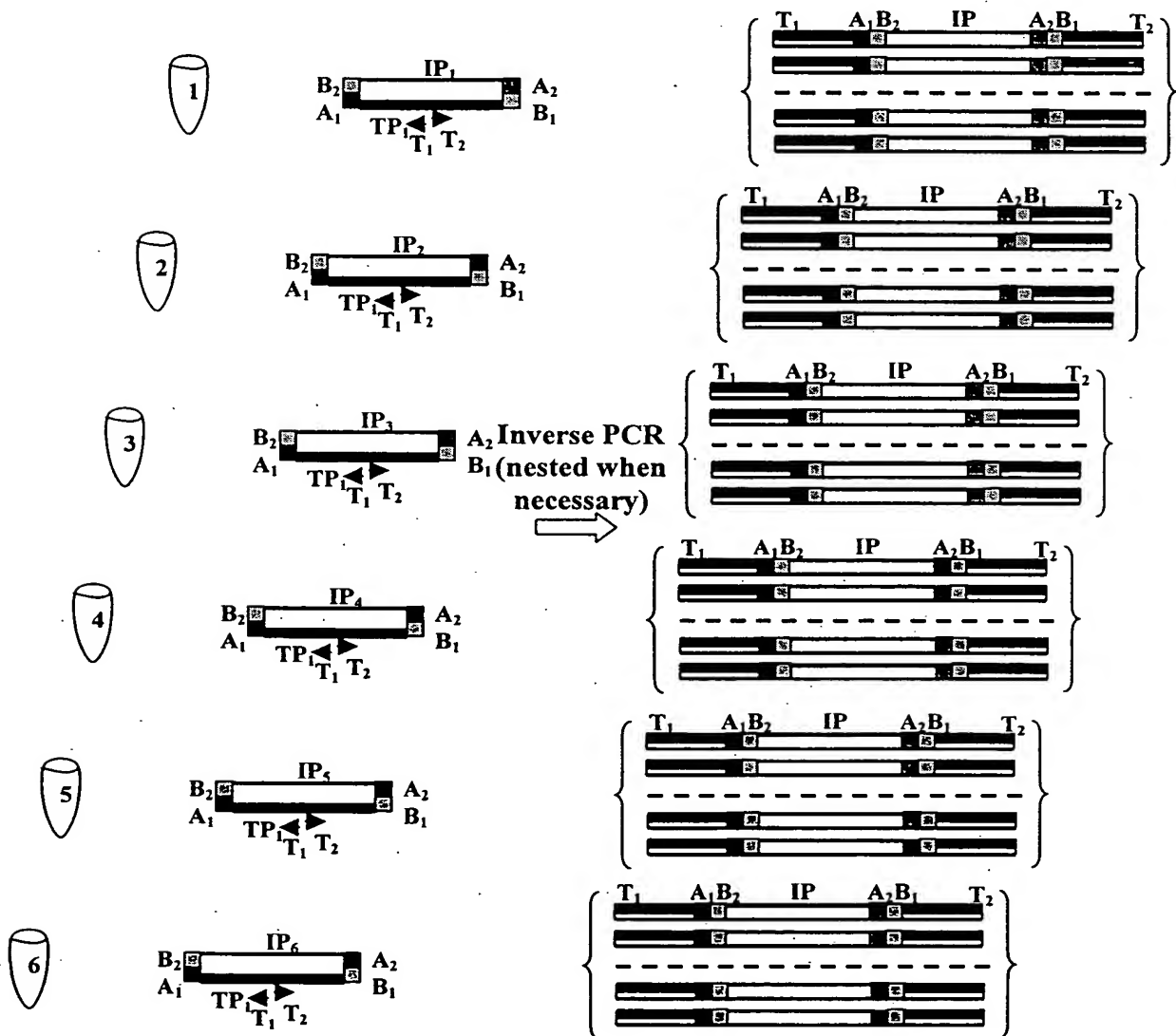


FIG. 17D



# A. SmartGenome DNA library I

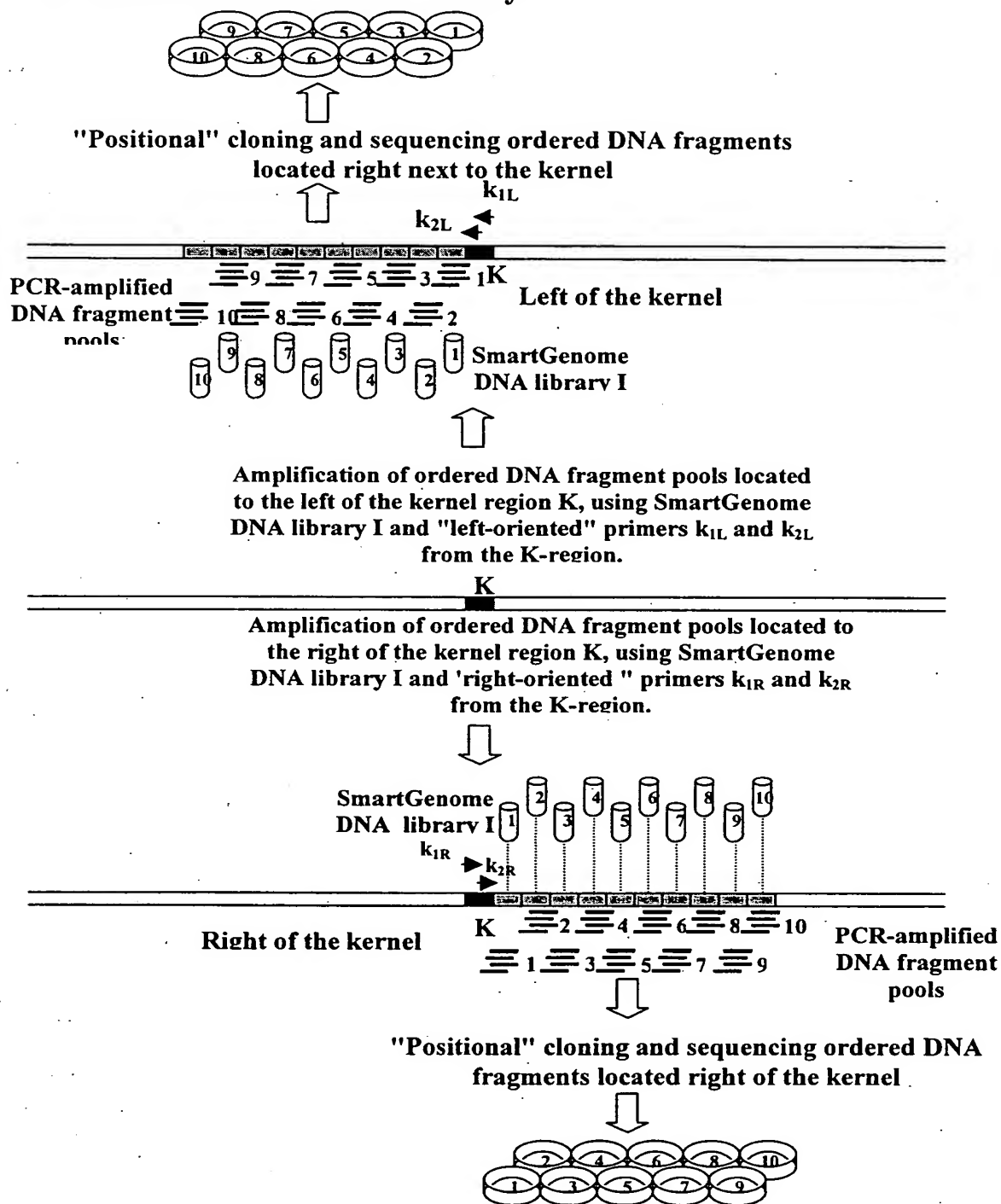
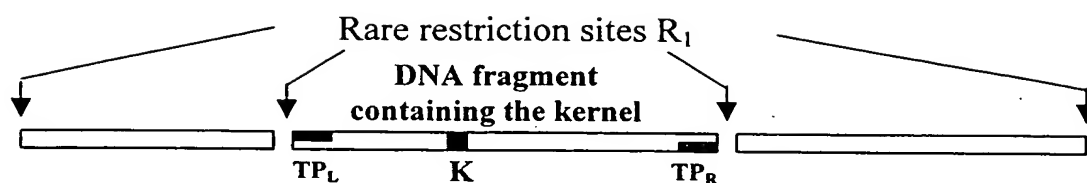
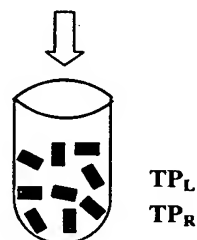


FIG. 18A



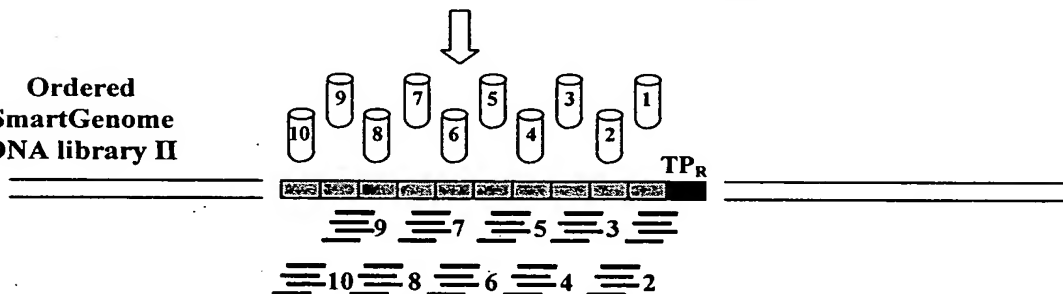
Amplification, isolation and sequencing of termini **TP<sub>L</sub>** and **TP<sub>R</sub>** of the DNA restriction fragment **R** with the kernel sequence using unordered "One-tube" SmartGenome DNA library II and primers **k<sub>1</sub>** and **k<sub>2</sub>** from the **K**-region

"One-tube"  
SmartGenome  
DNA library II



Amplification of the ordered internal DNA molecules located within the same DNA restriction fragment **R** using ordered SmartGenome DNA library II and primers from the terminal regions

Ordered  
SmartGenome  
DNA library II



"Positional" cloning and sequencing ordered DNA molecules within the restriction fragment containing the kernel

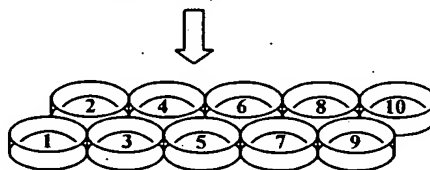
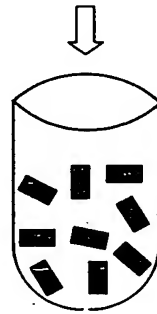
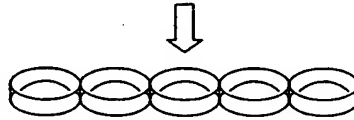


FIG. 18B

**Step 1** Linear amplification of termini ( $TP_L$  and  $TP_R$ ) of all DNA restriction fragments using unordered "One-tube" SmartGenome DNA library II and adaptor-primers  $A_1$  and  $B_1$  (see Fig. 17A)



Cloning and sequencing the termini of all DNA restriction fragments



Database of terminal PENTamer DNA sequences

**Step 2** Amplification of the ordered internal DNA fractions located within the DNA restriction fragments using ordered SmartGenome DNA library II and primers from the terminal regions

**Step 3** Amplification, isolation and sequencing of the "linking" DNA fractions using ordered SmartGenome DNA library I and primers within the adjacent terminus region

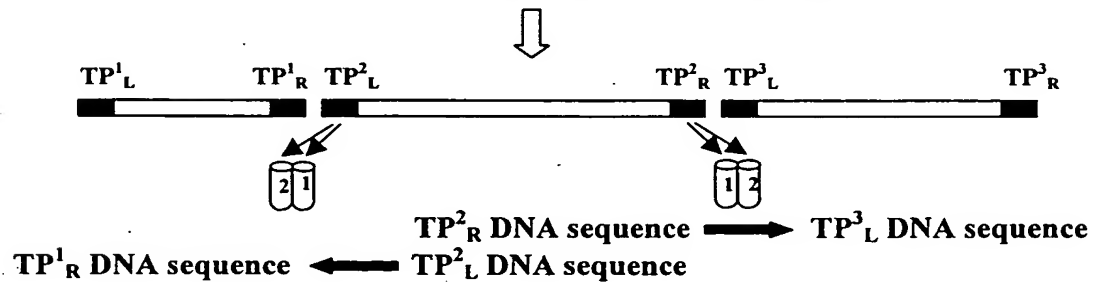


FIG. 18C

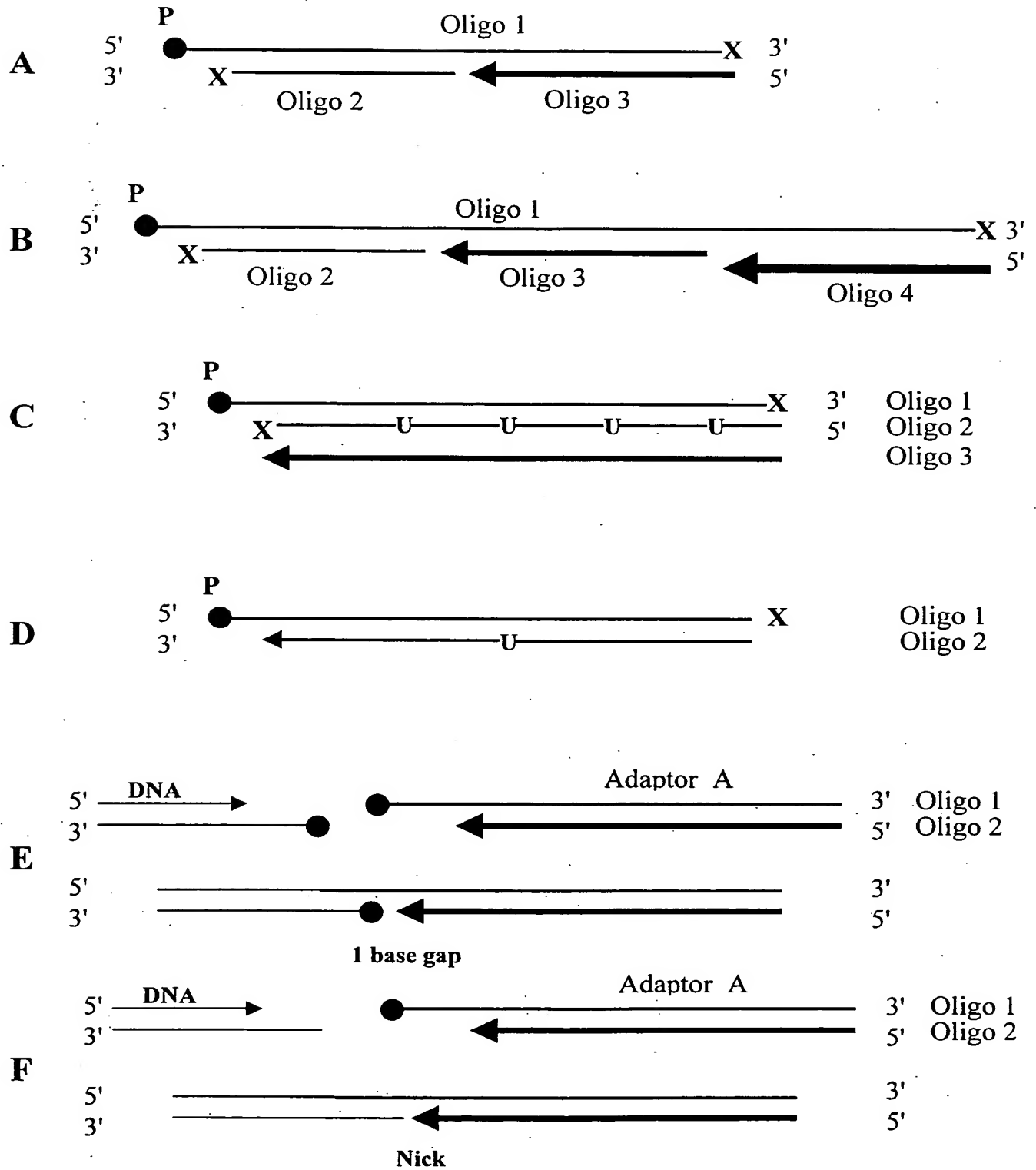


FIG. 19

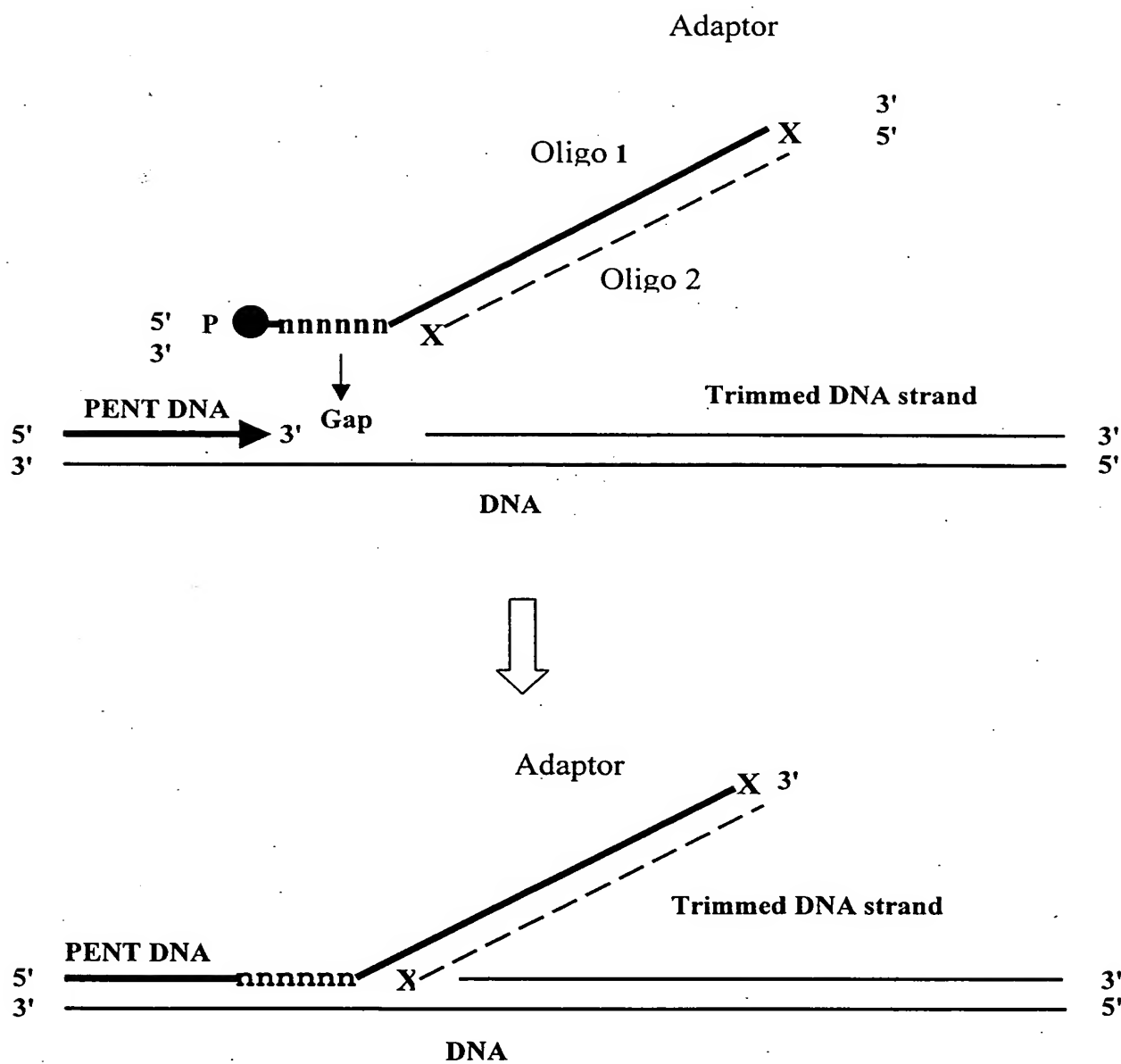


FIG. 20

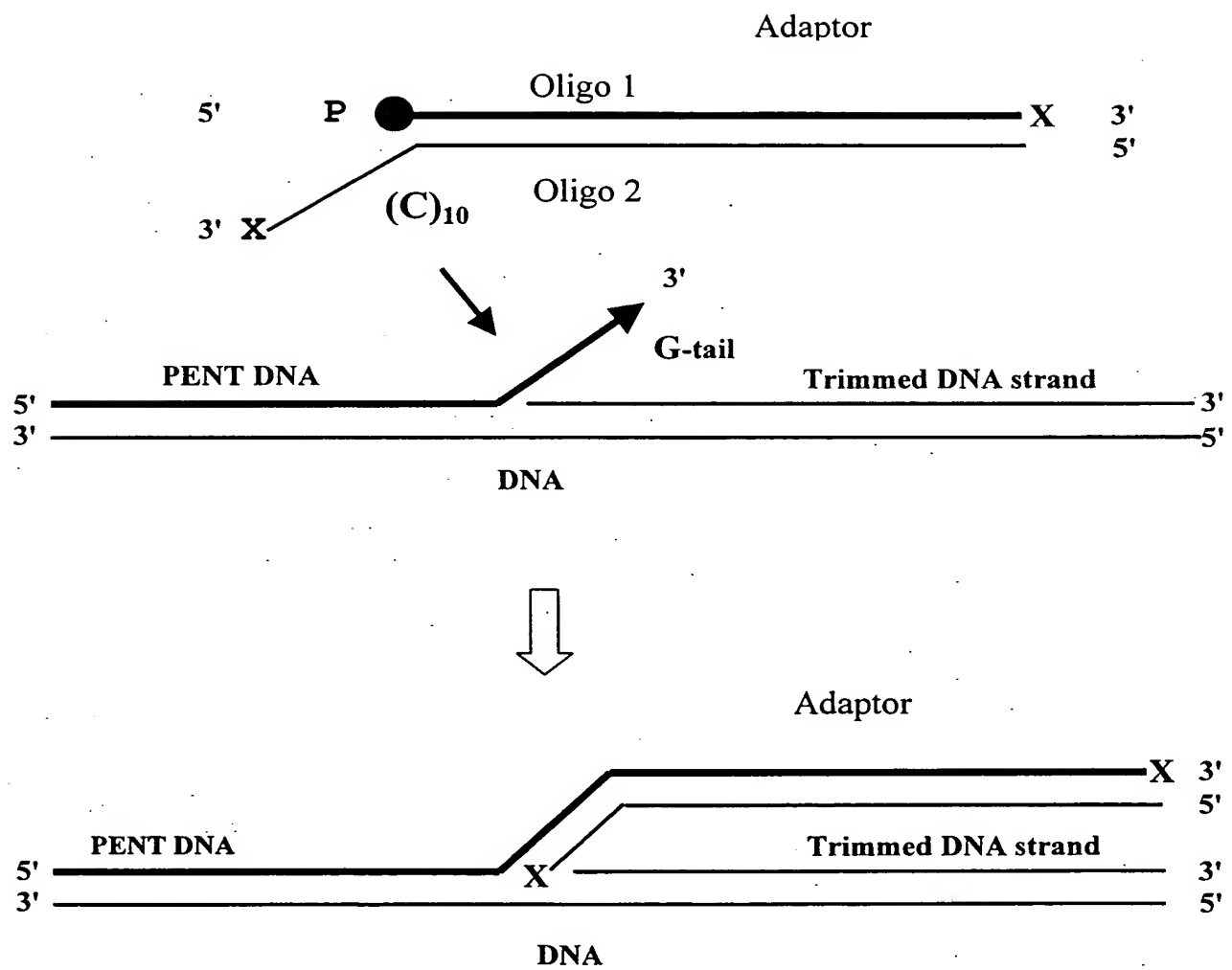


FIG.21

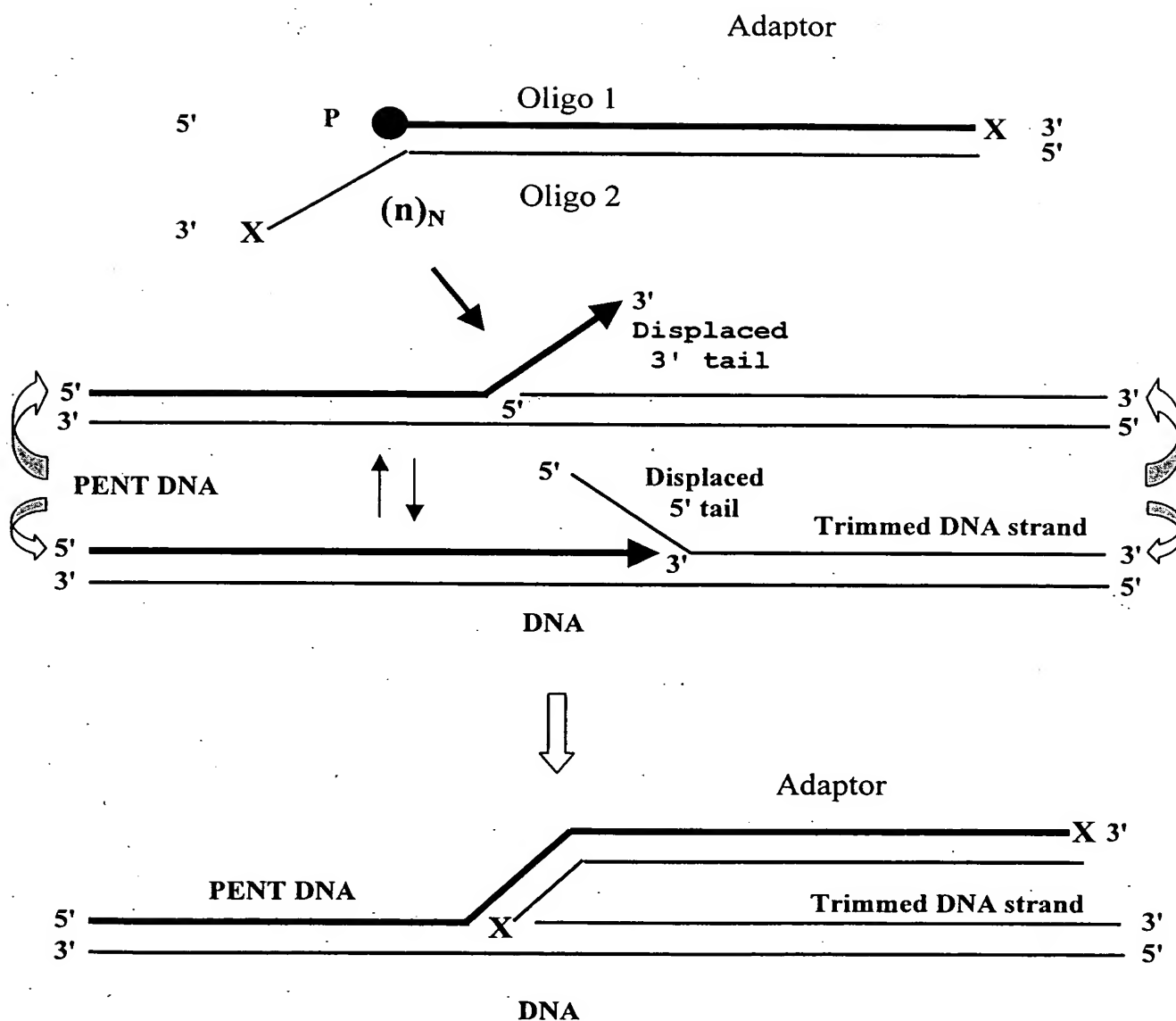


FIG. 22

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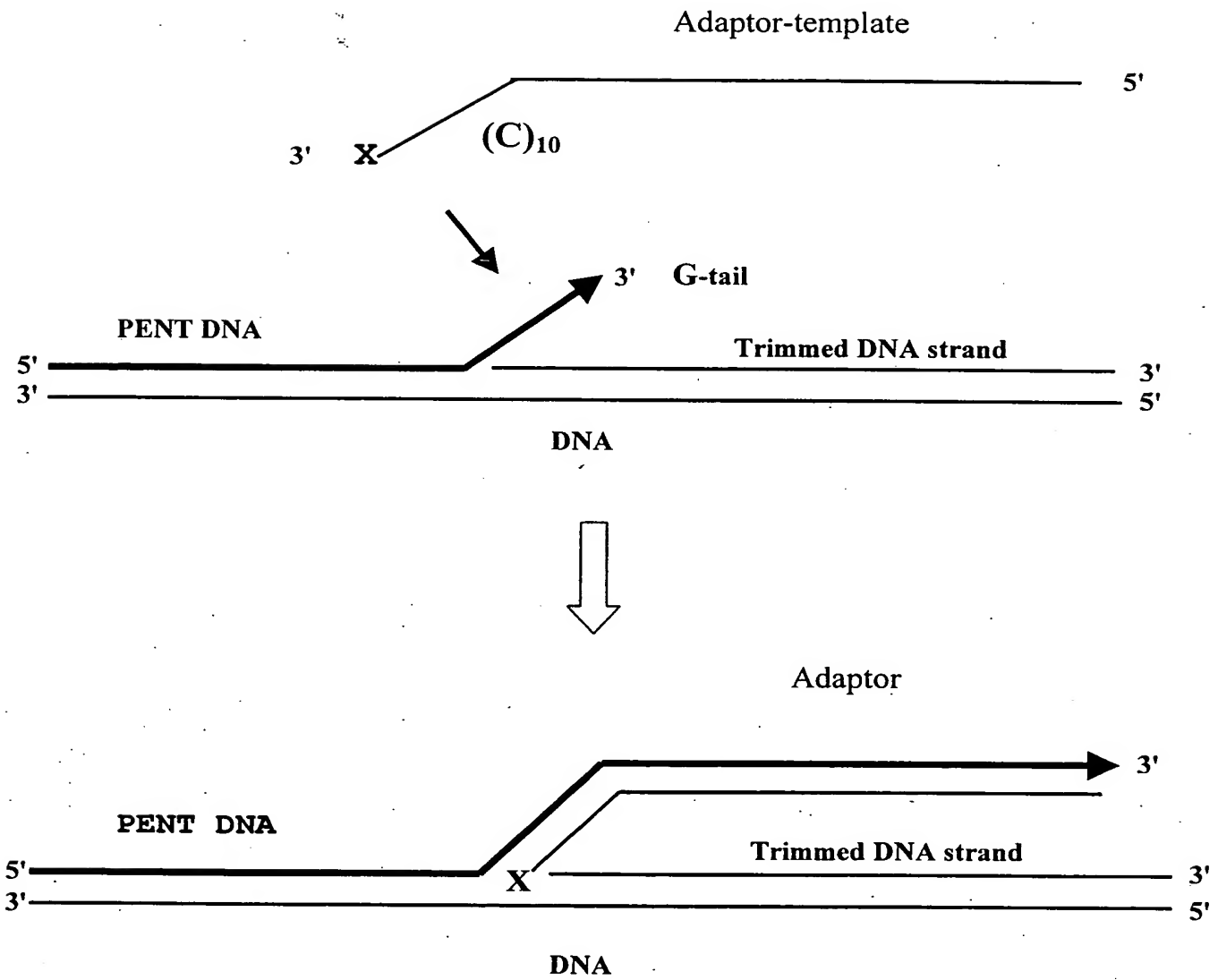


FIG. 23



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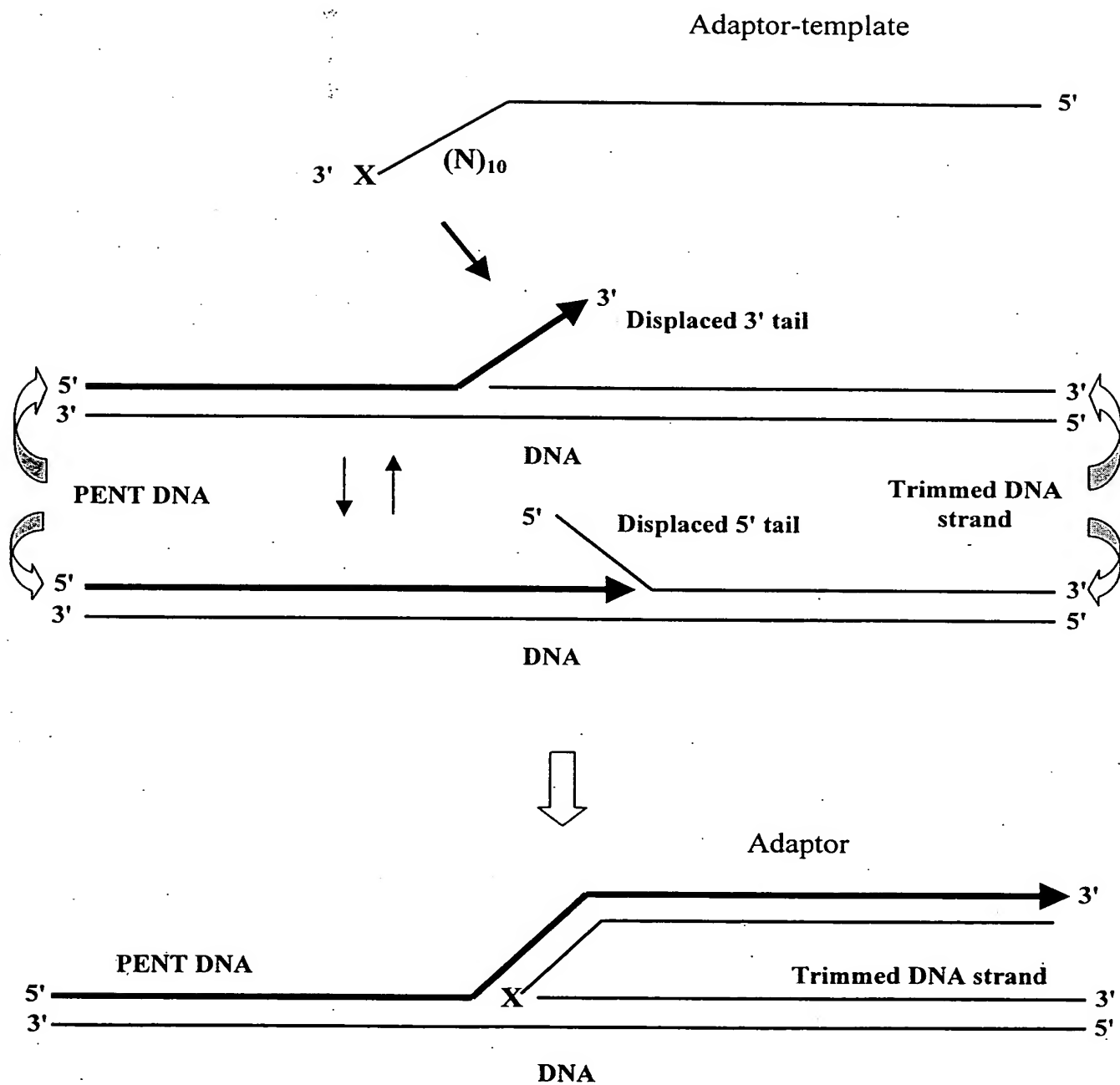


FIG. 24

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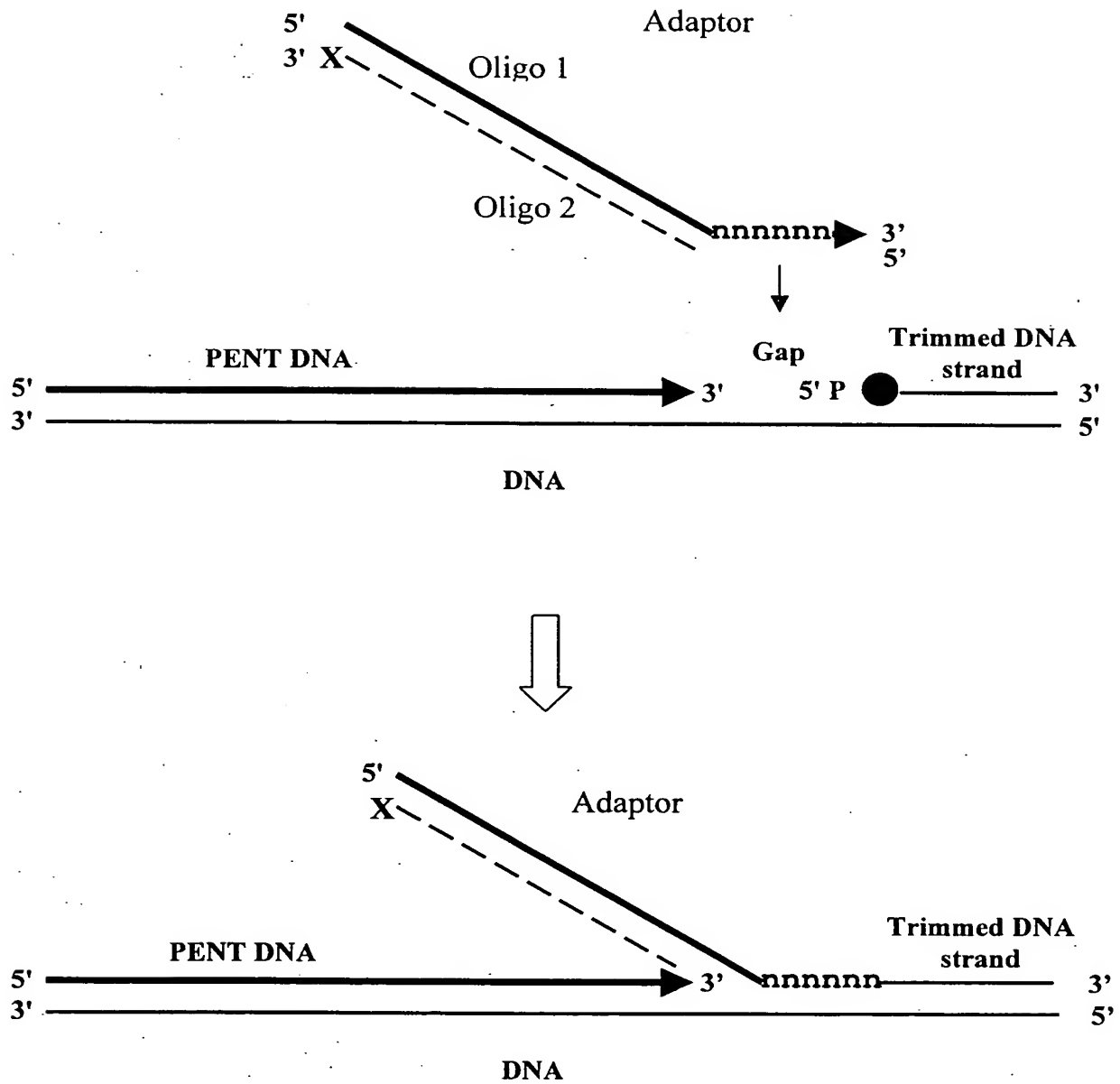


FIG. 25

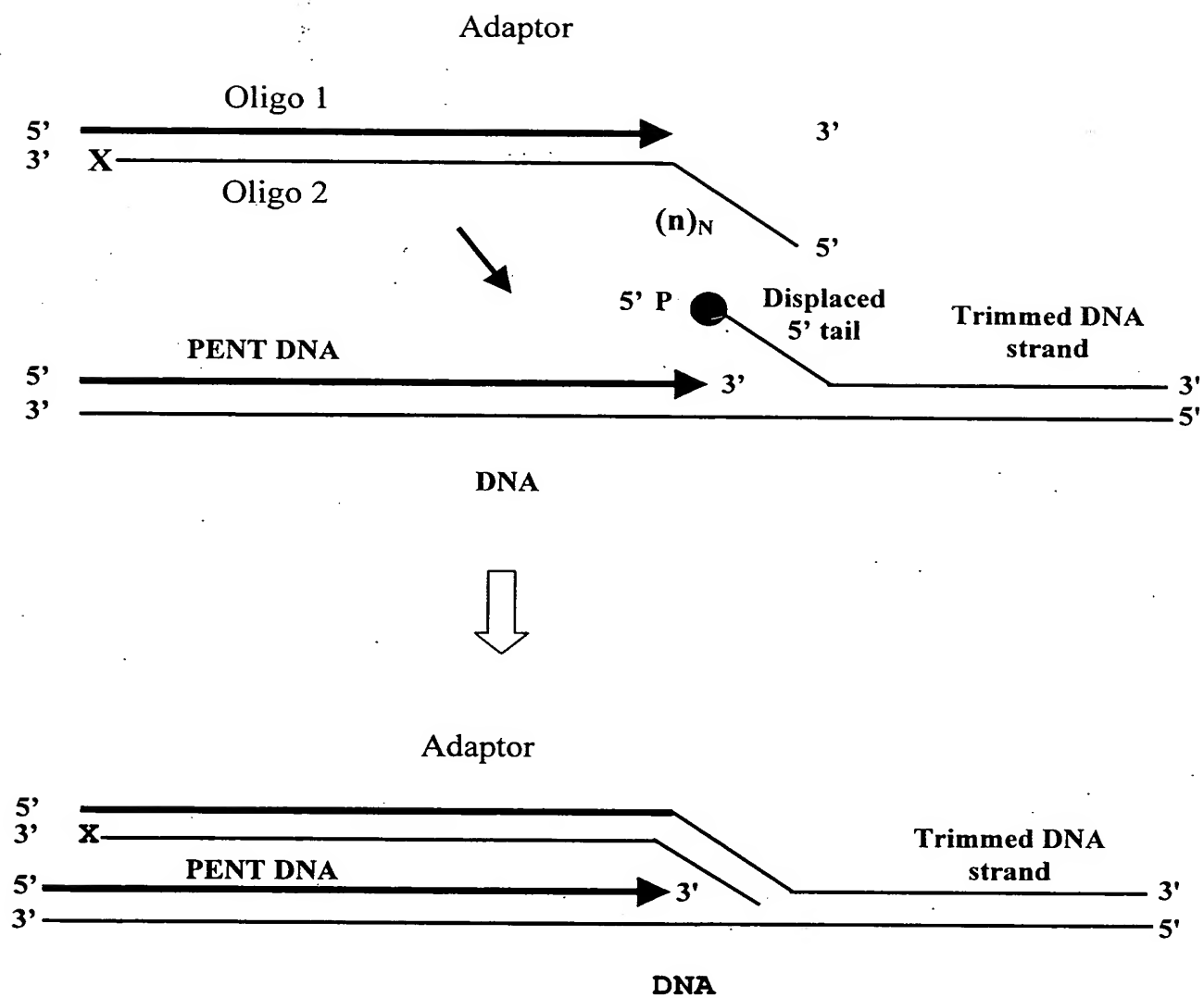


FIG. 26

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**A**

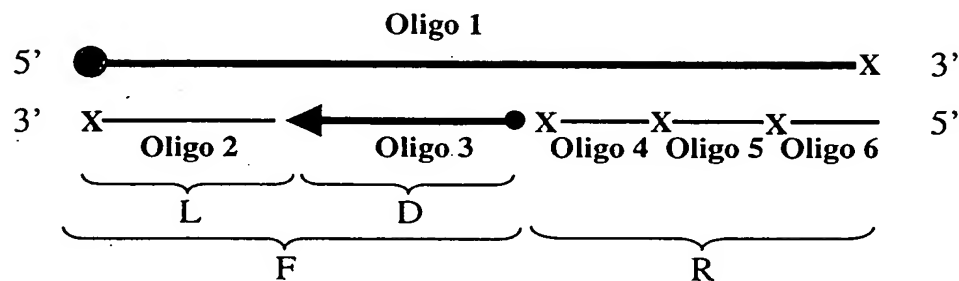
<b>F</b>	<b>R</b>
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**B**

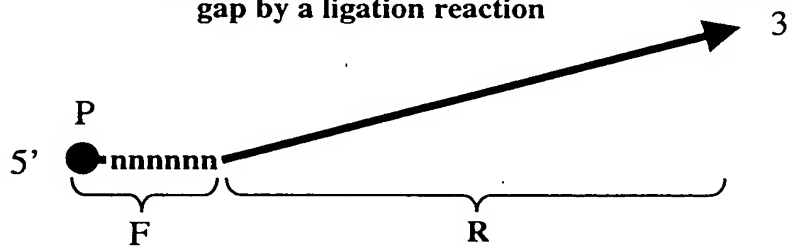
<b>L</b>	<b>I</b>	<b>R</b>
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FIG. 27

**A Up-stream terminus-attaching nick-translation recombination adaptor RA**



**B Down-stream nick-attaching recombination adaptor RB-3' (I) targeted to a gap by a ligation reaction**



**C Down-stream nick-attaching recombination adaptor RB-3' (II) targeted to a poly-G tail by a ligation reaction**

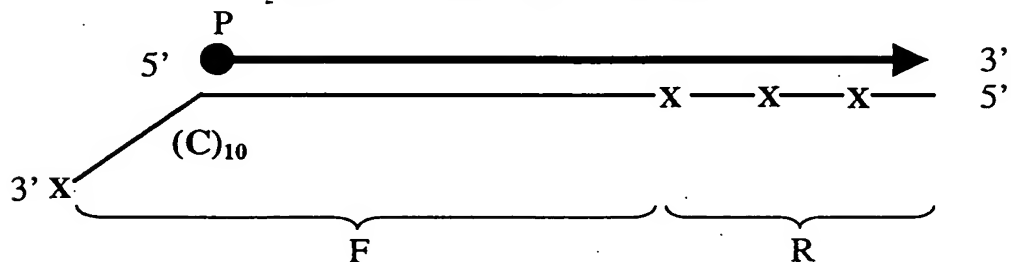
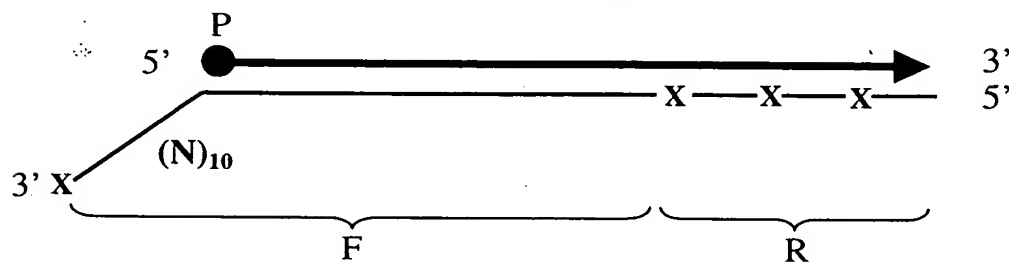
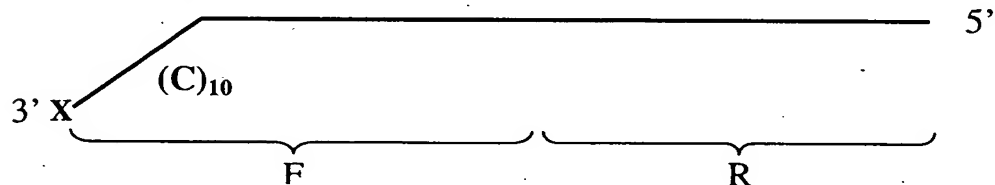


FIG. 28A

- D** Down-stream nick-attaching recombination adaptor RB-3' (III) targeted to a displaced 3' DNA tail by a ligation reaction



- E** Down-stream nick-attaching recombination adaptor RB-3' (IV) targeted to a poly-G tail as a template for a polymerization-extension reaction



- F** Down-stream nick-attaching recombination adaptor RB-3' (V) targeted to a displaced 3' DNA tail as a template for a polymerization-extension reaction

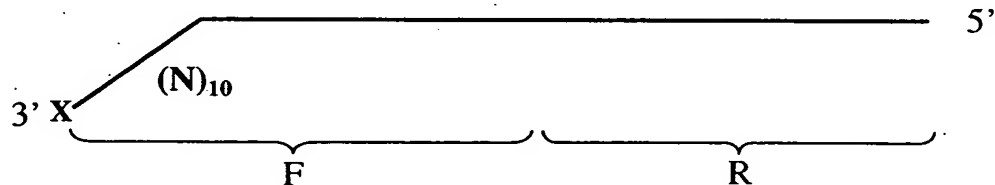
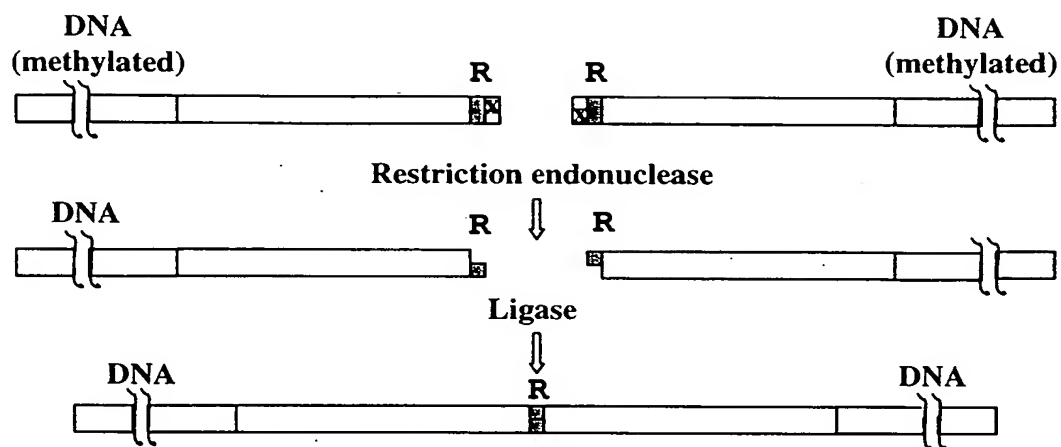


FIG. 28B

## A RecAdaptors-Class I



## B RecAdaptors-Class II

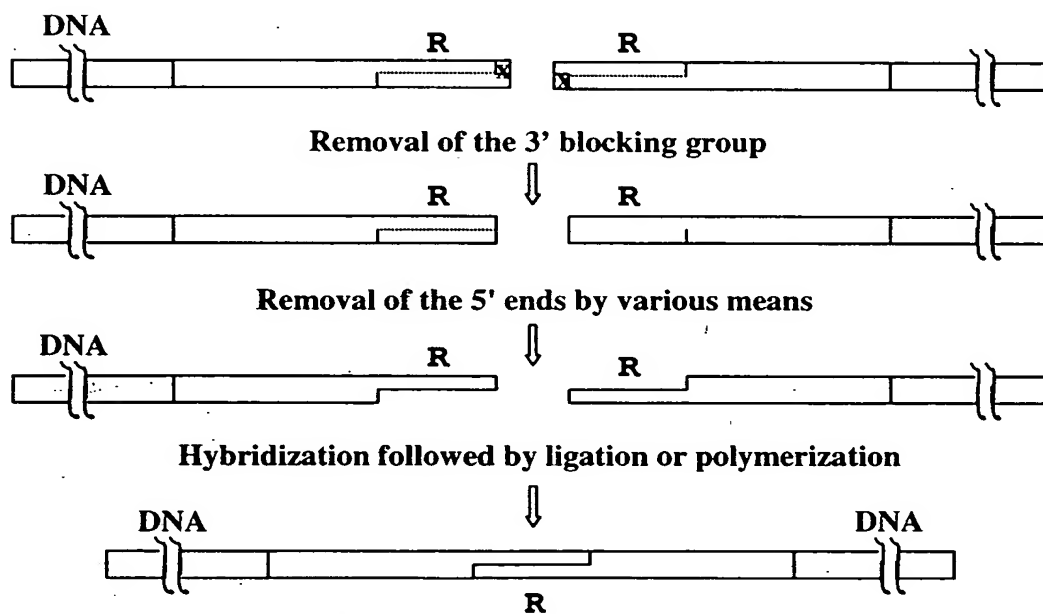


FIG. 29

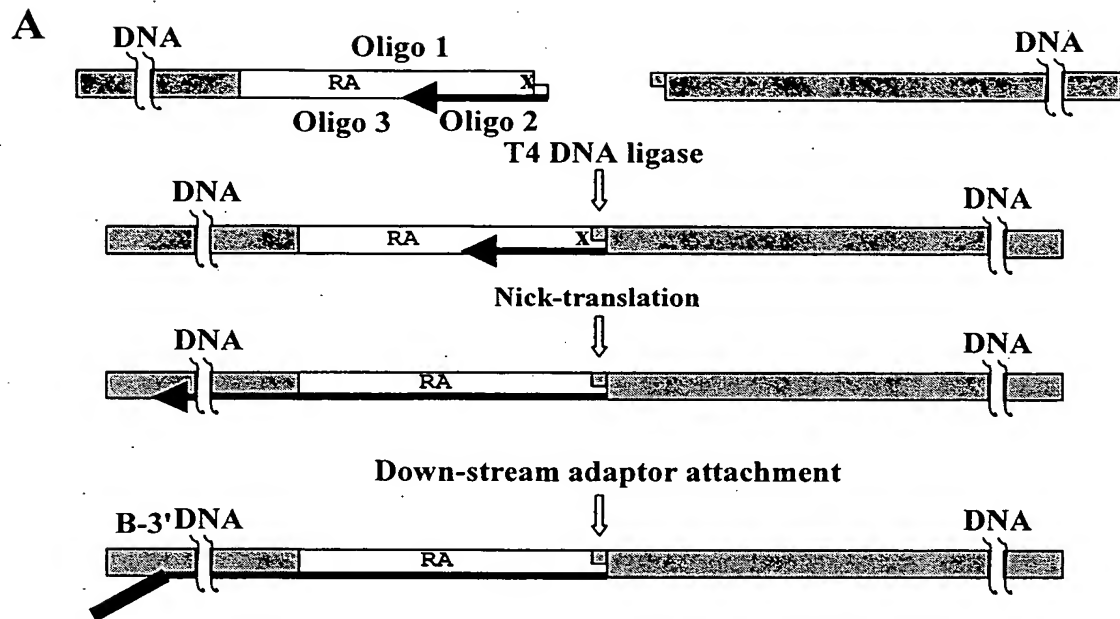
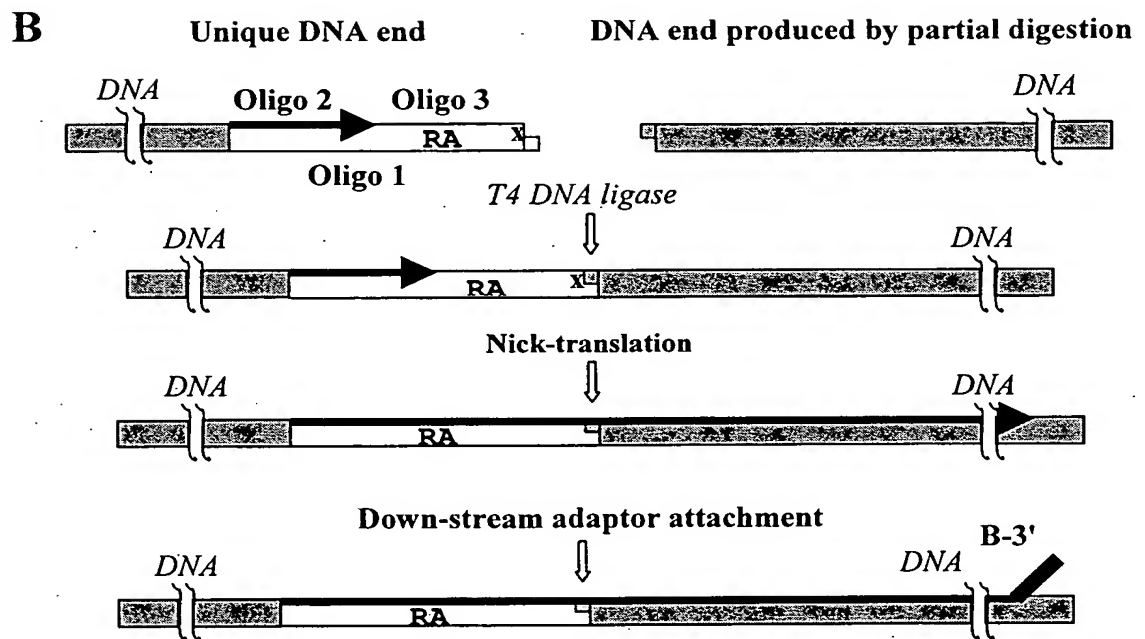


FIG. 30A





**FIG. 30B**

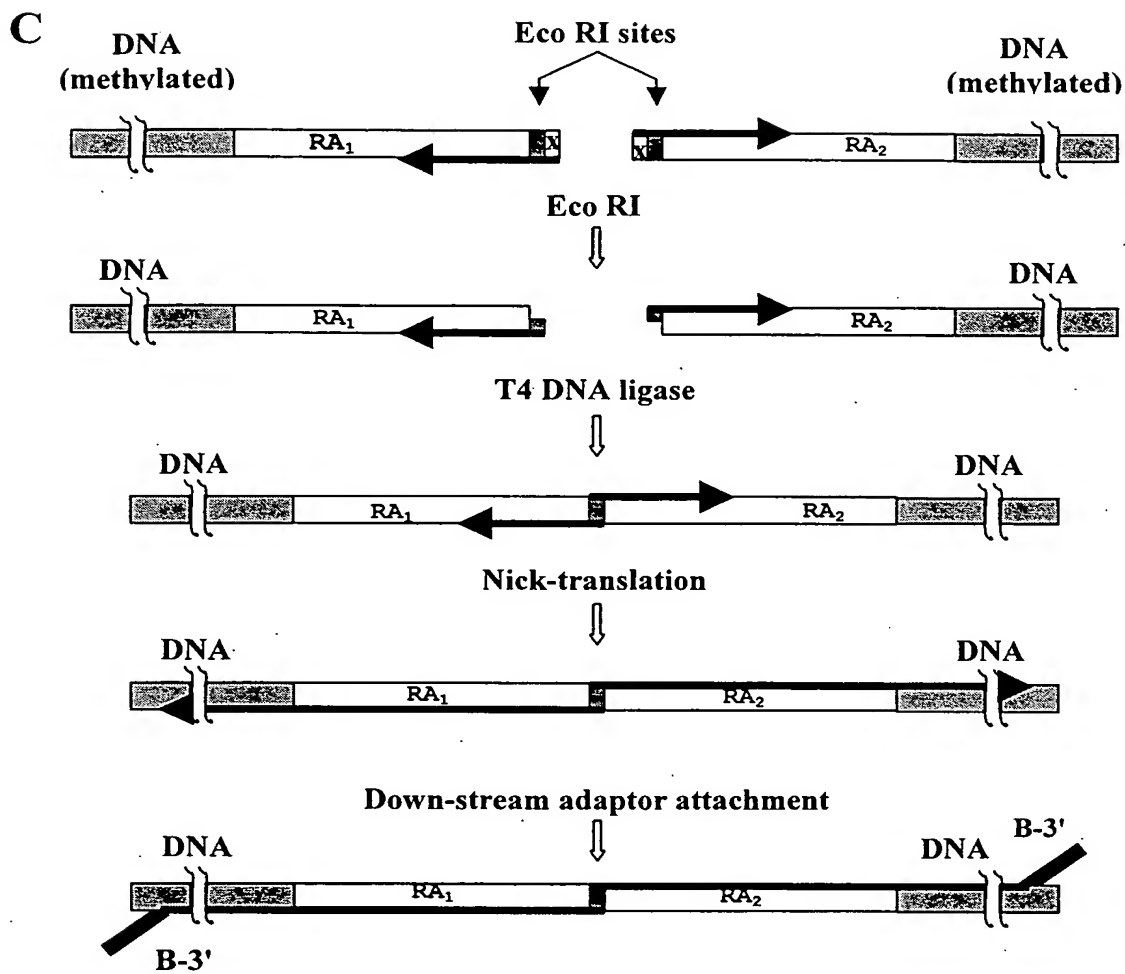


FIG. 30C

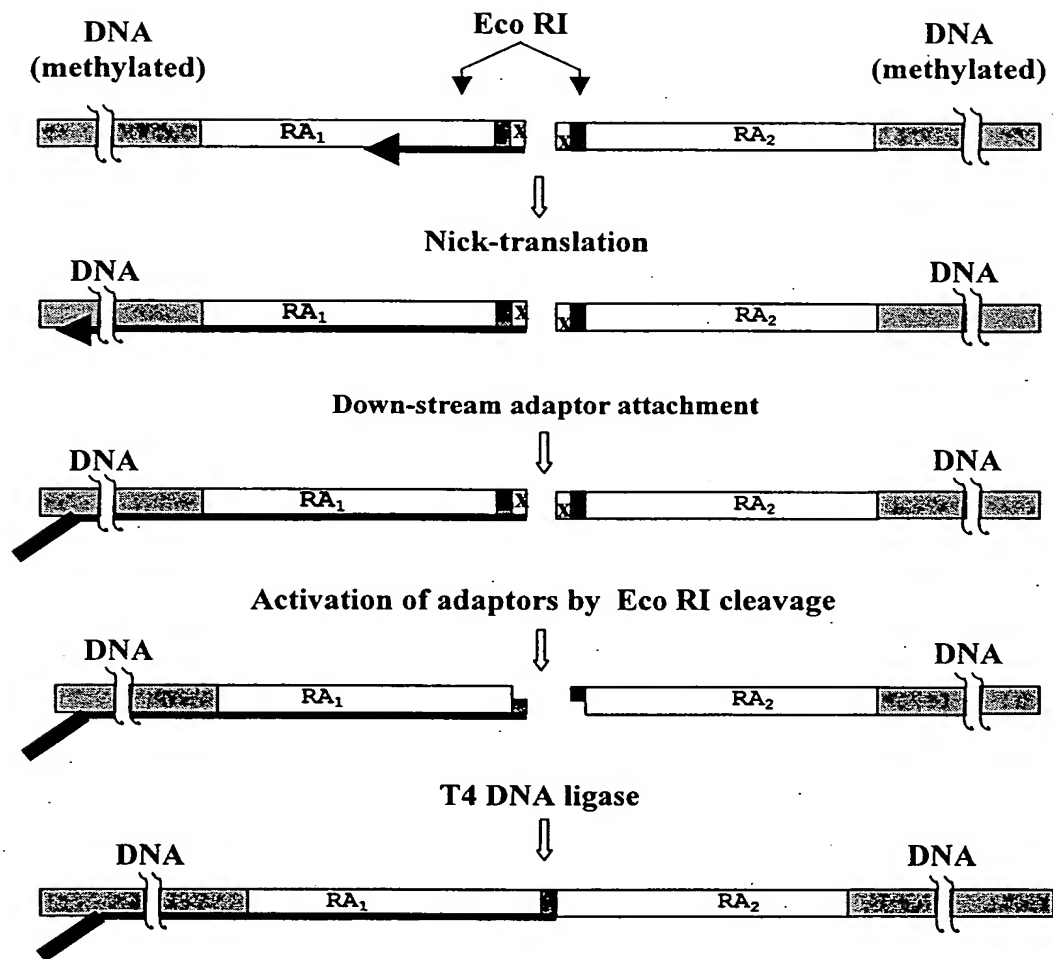
**D**

FIG. 30D

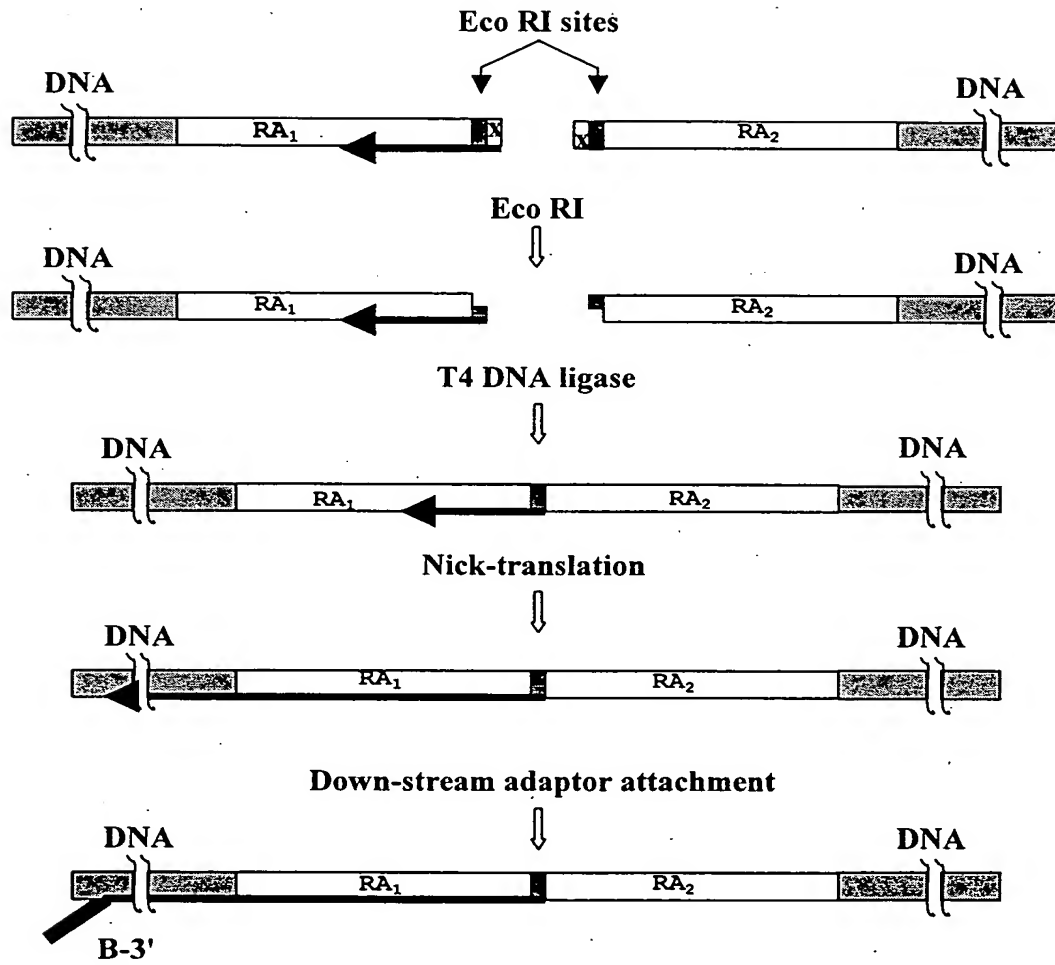
**E**

FIG. 30E

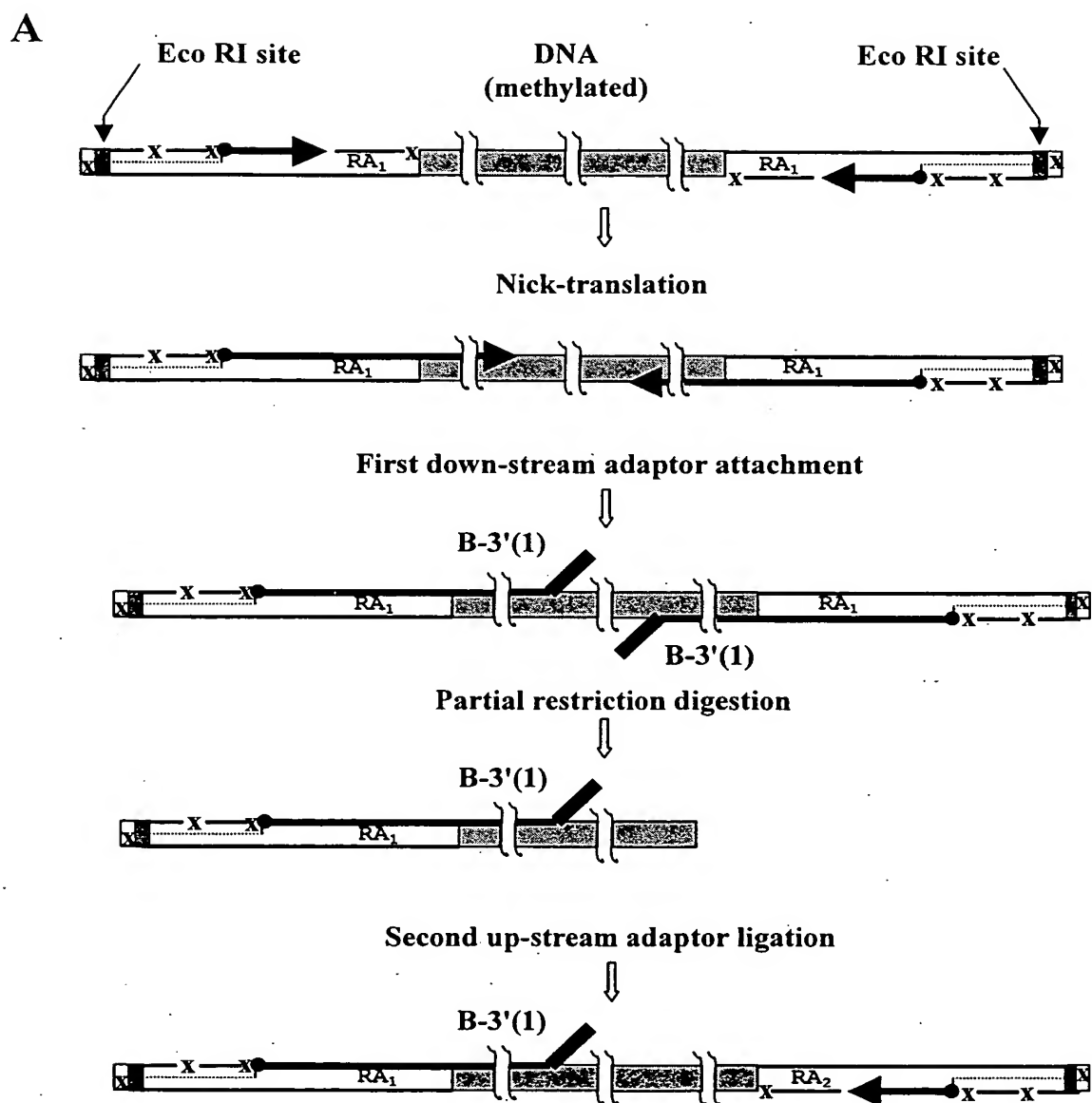


FIG. 31A

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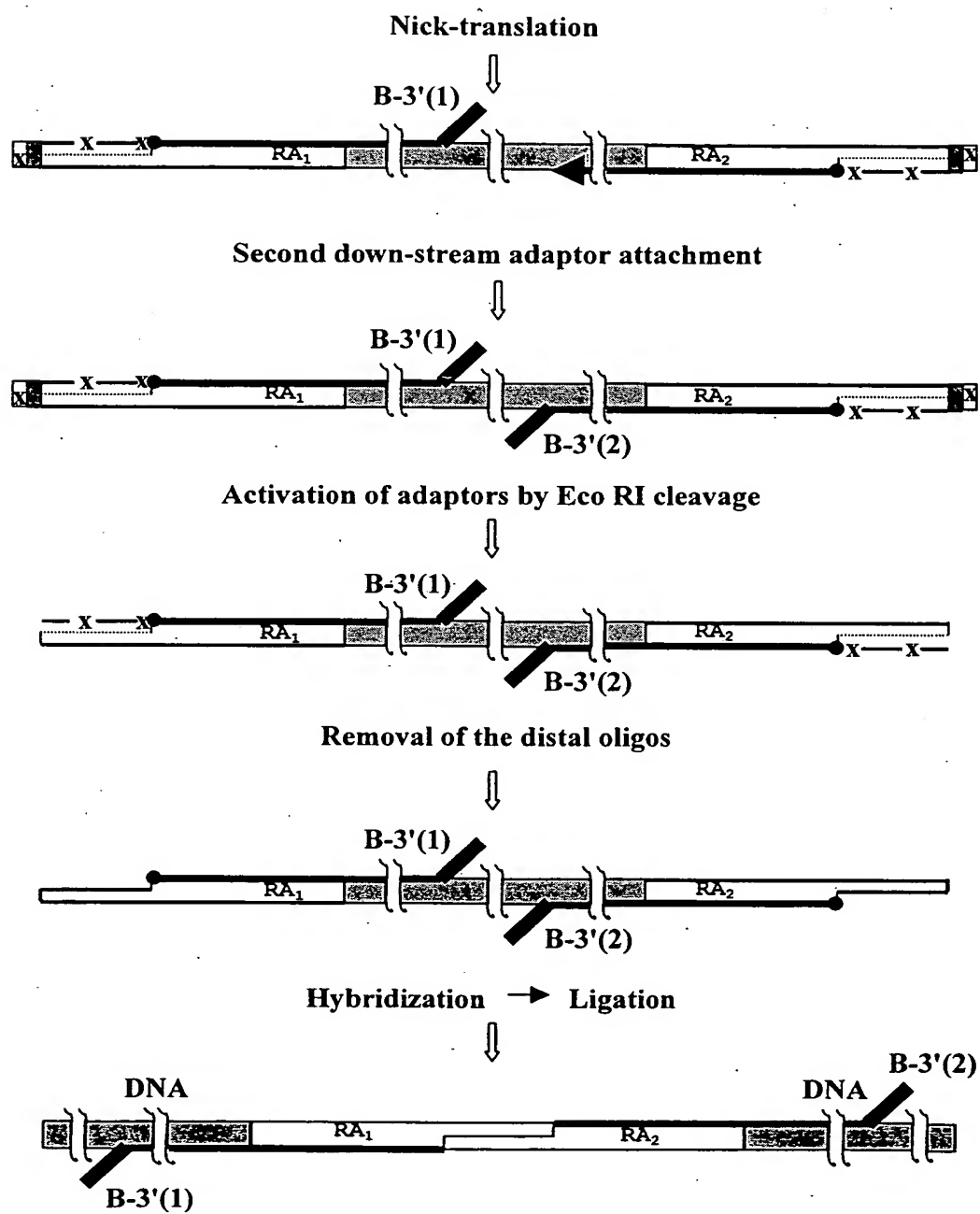


FIG. 31B

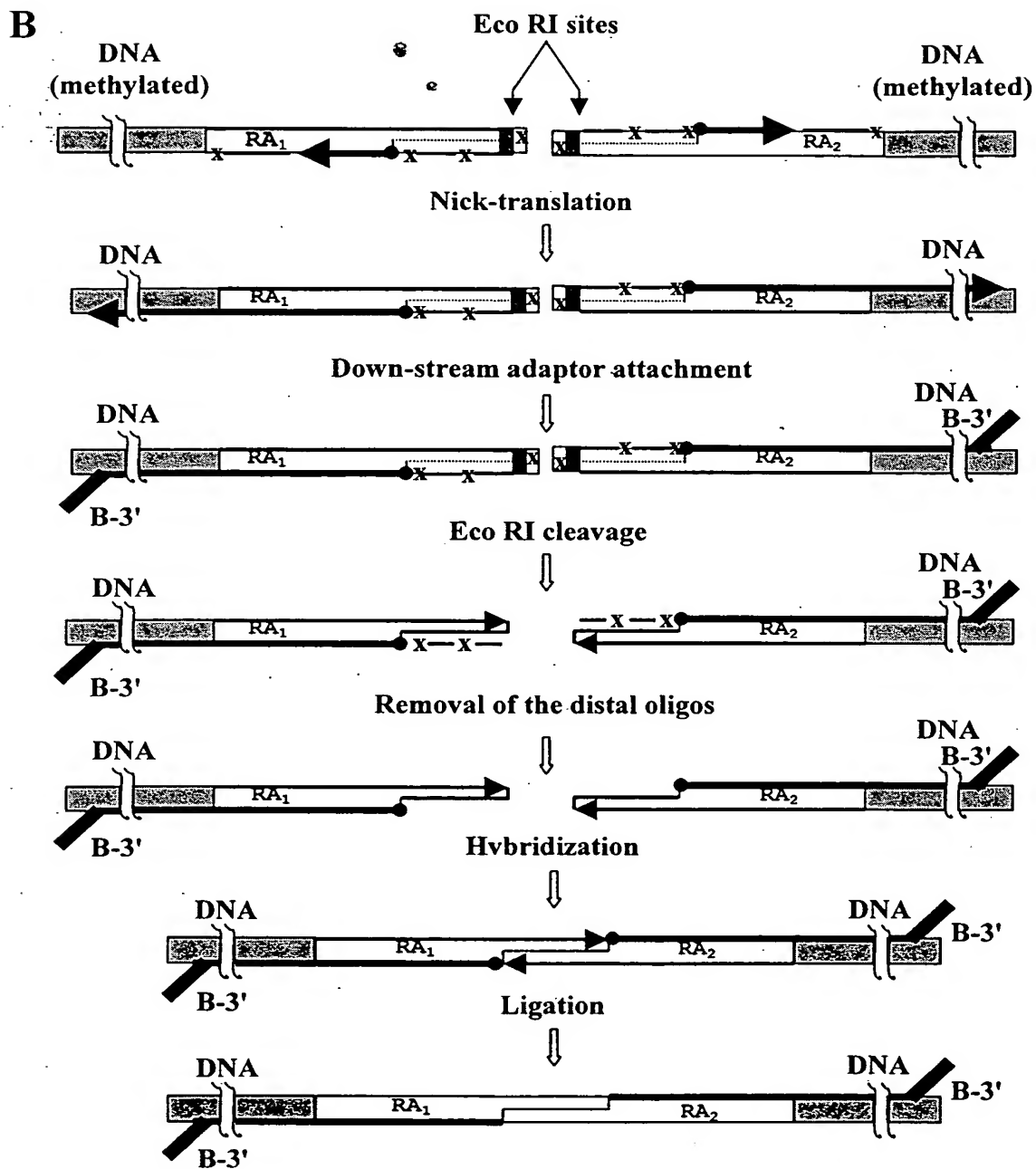


FIG. 31C

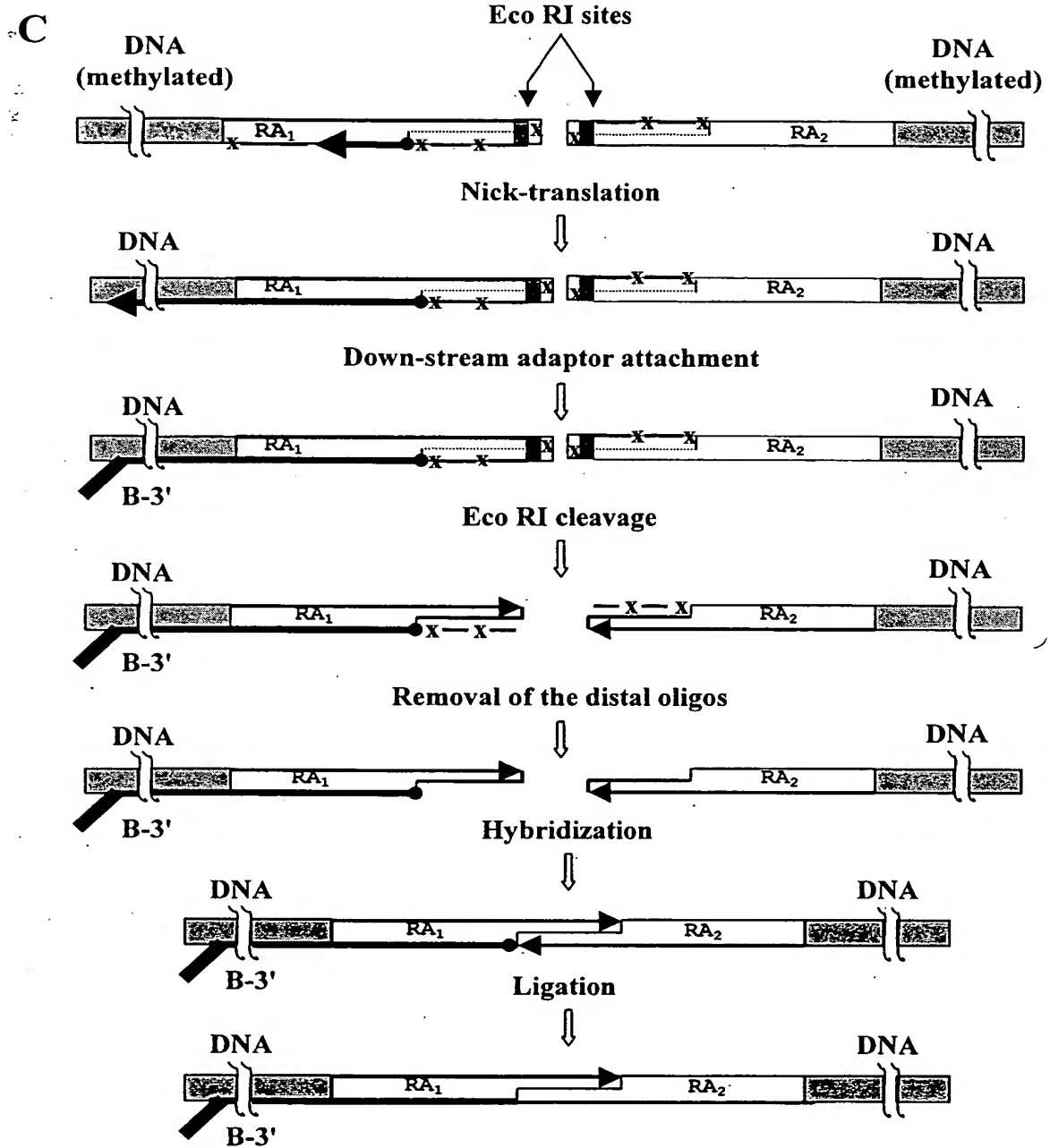


FIG. 31D



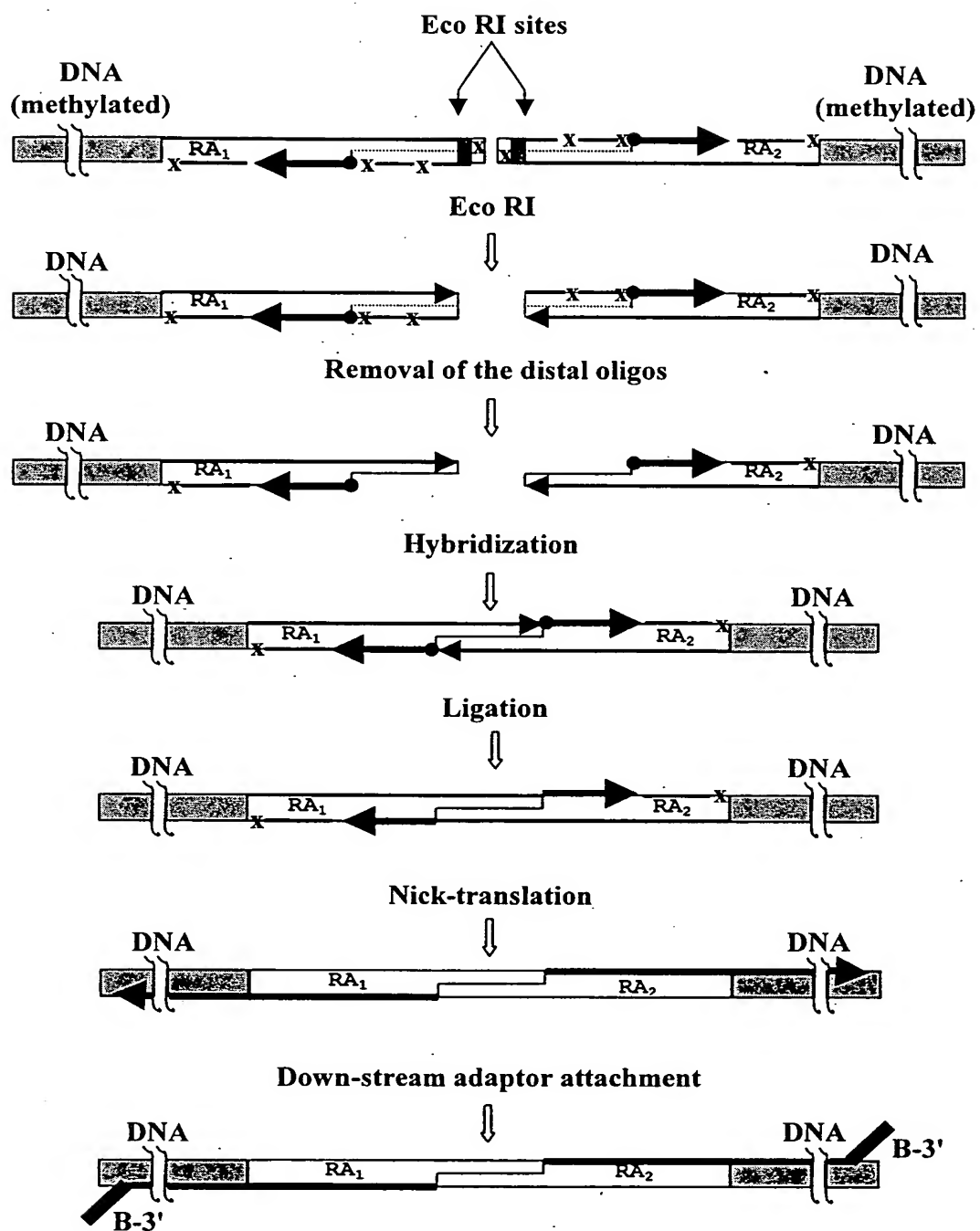
**D**

FIG. 31E

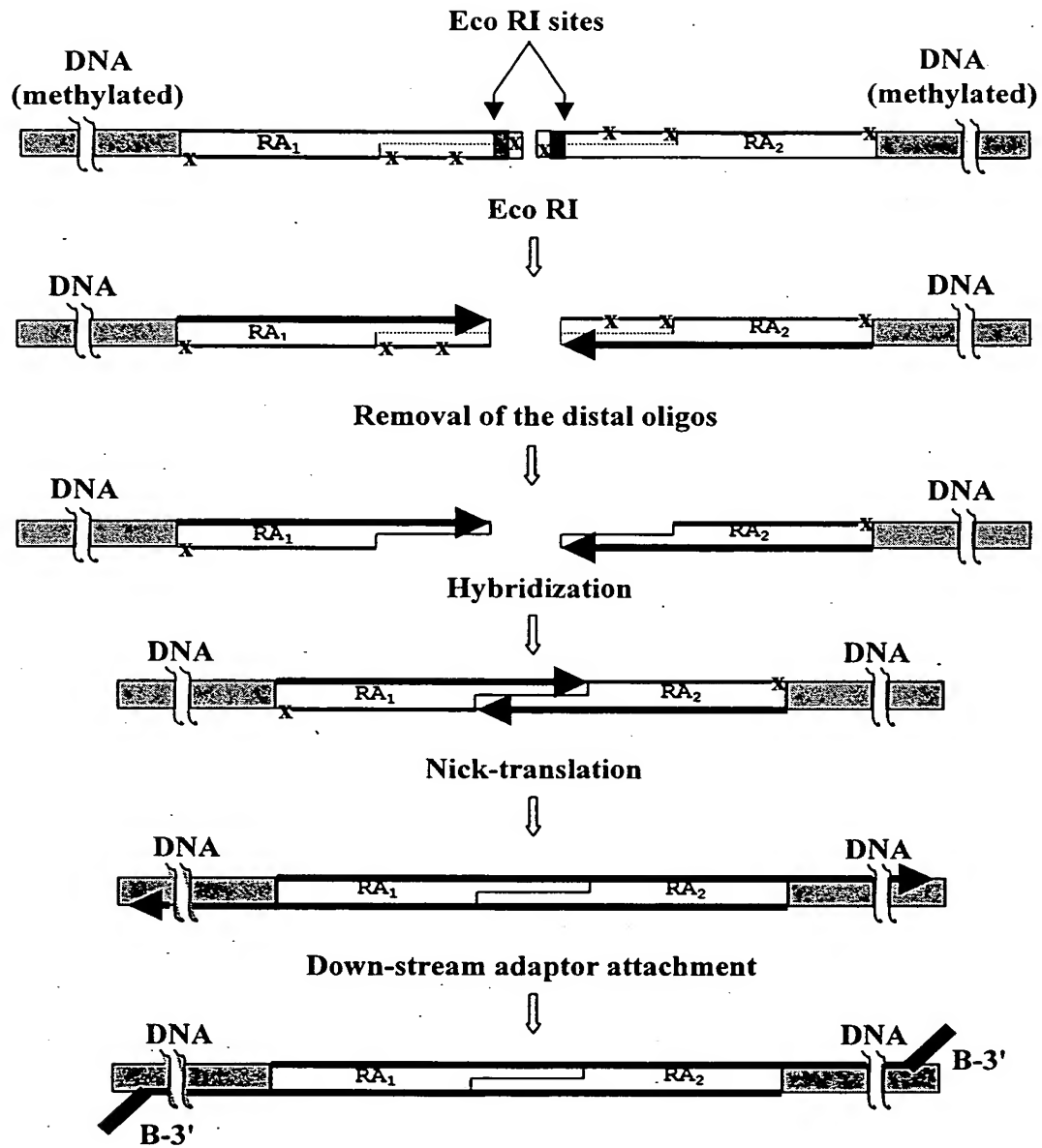


FIG. 32

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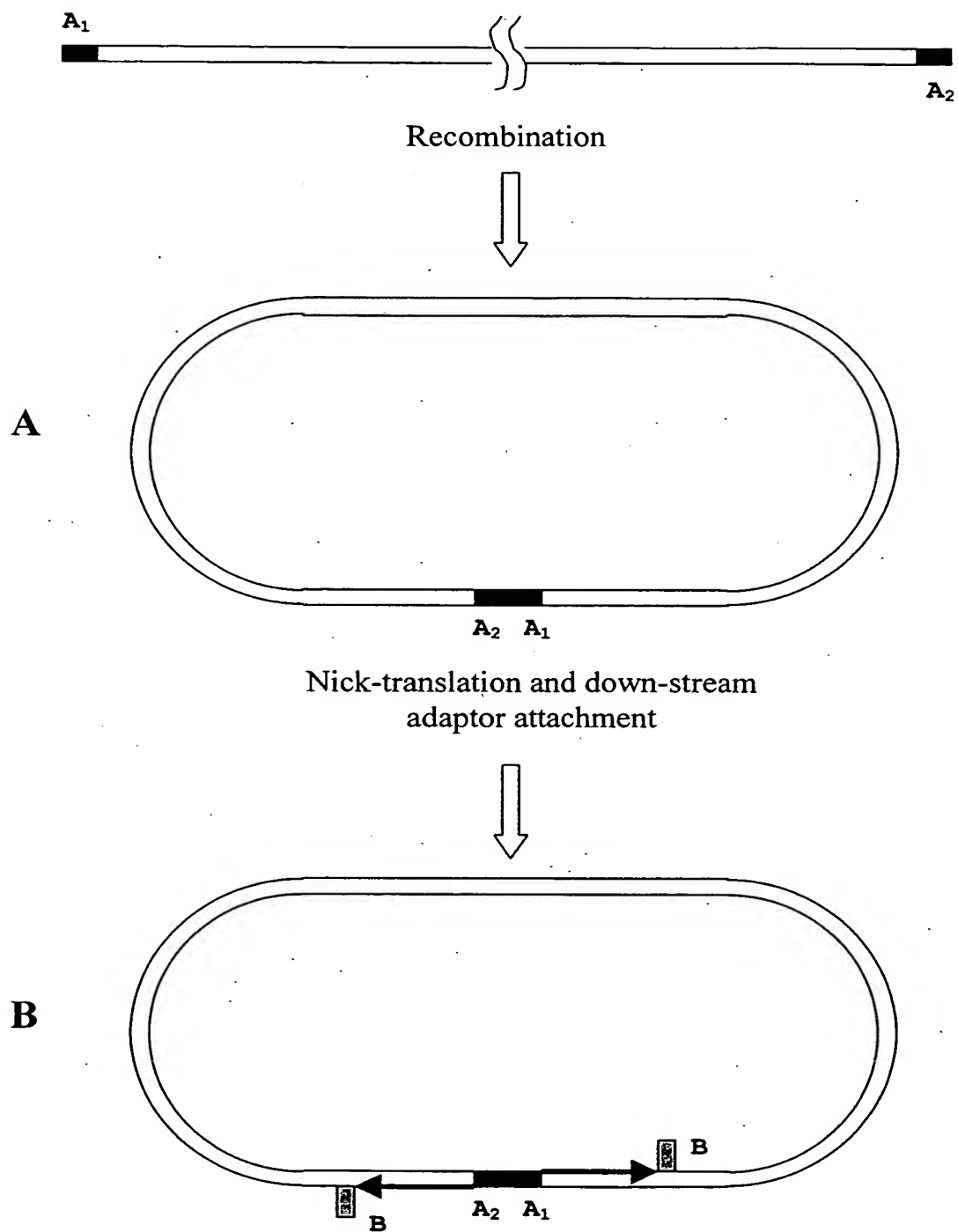


FIG. 33

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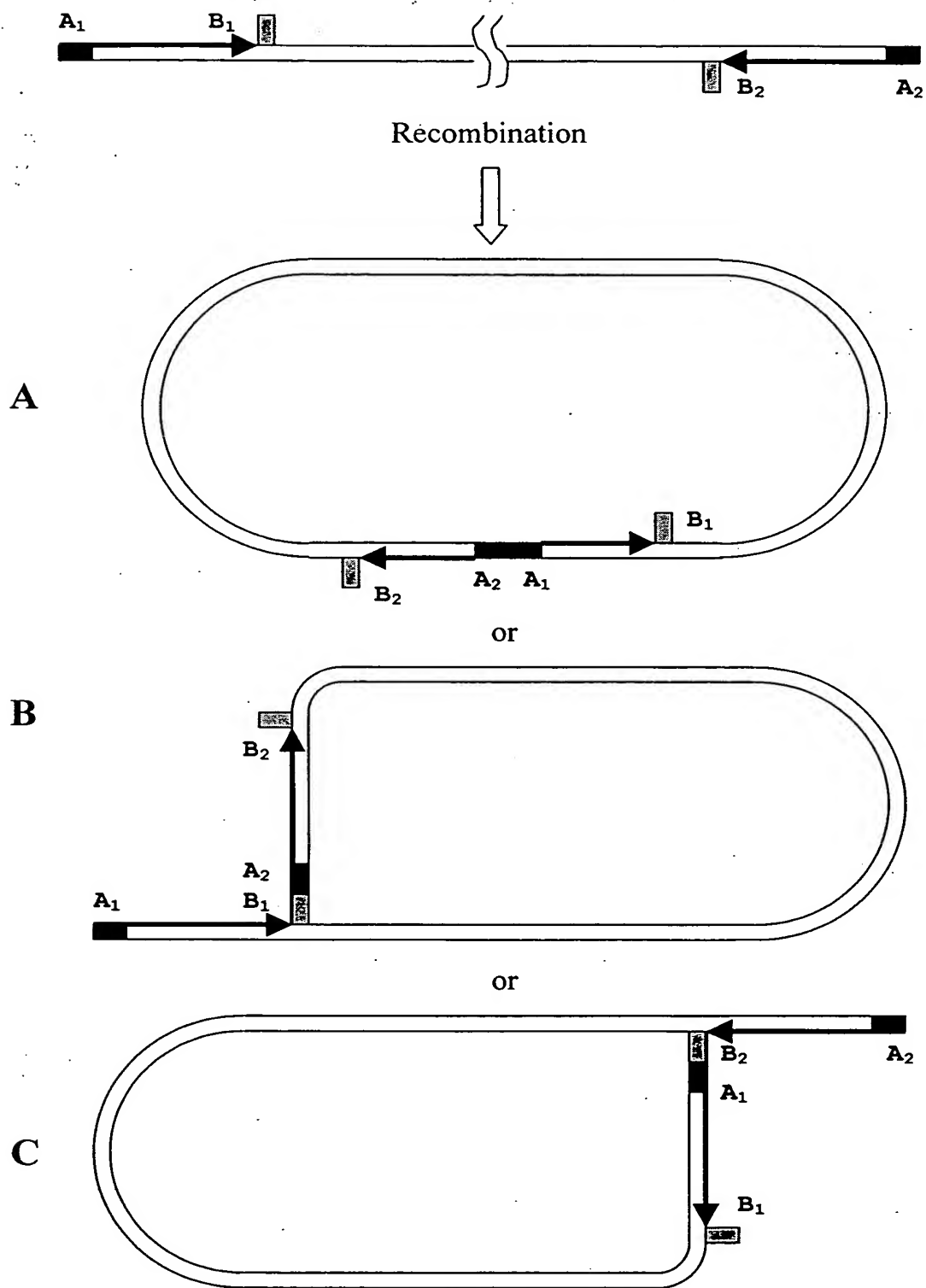
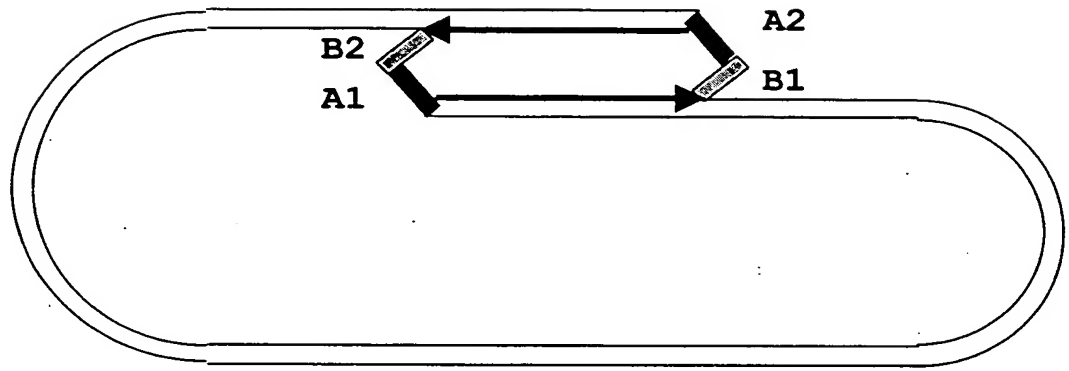


FIG. 34A

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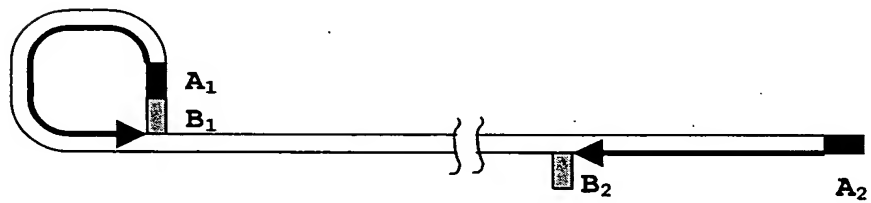
or

**D**



or

**E**



or

**F**

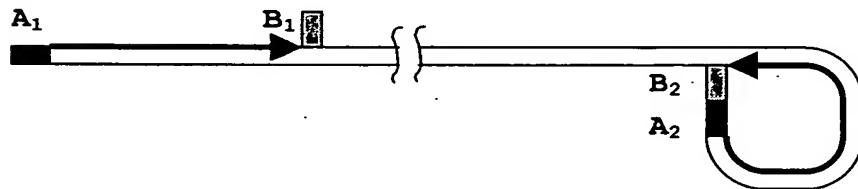


FIG. 34B

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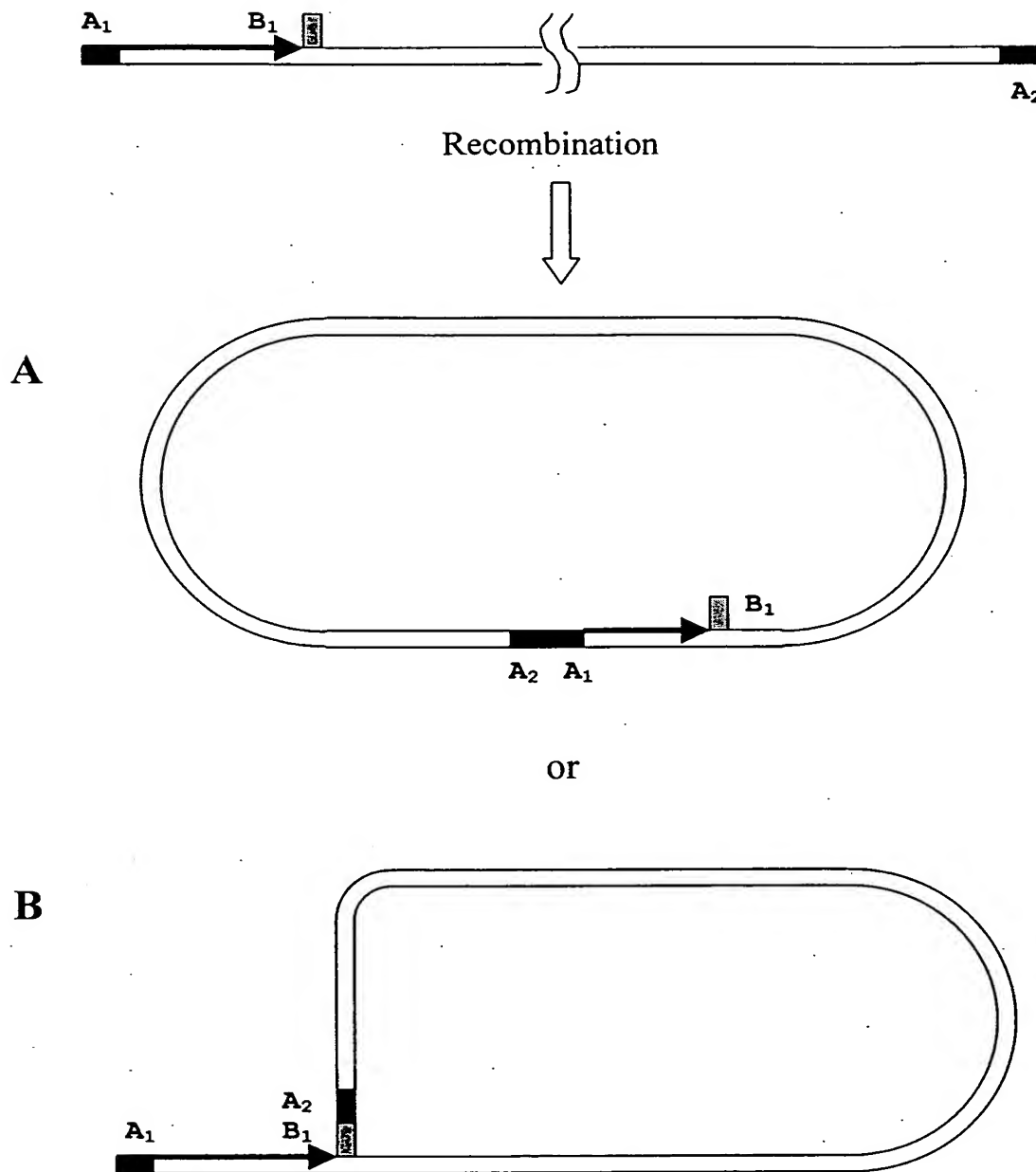


FIG. 35

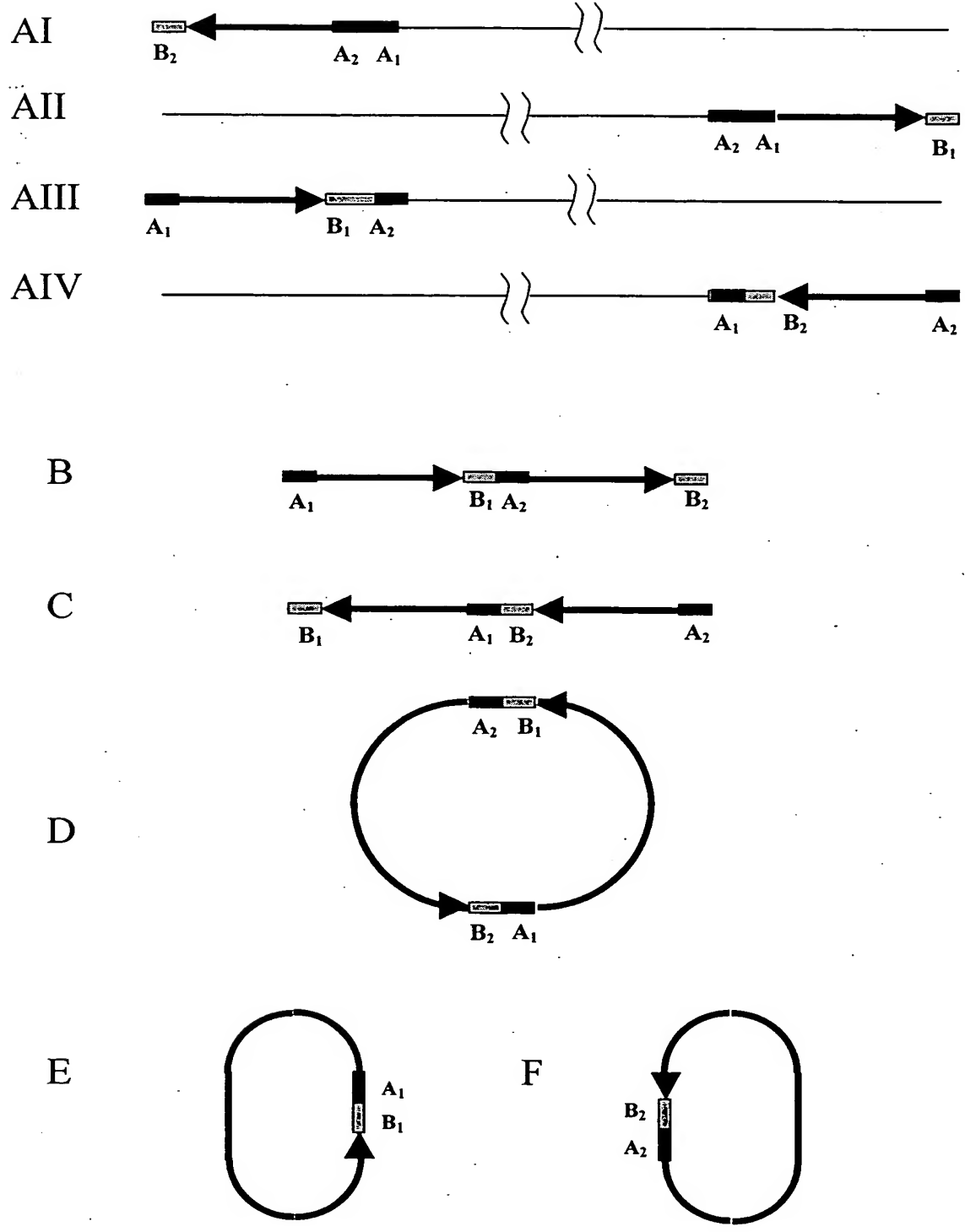


FIG. 36

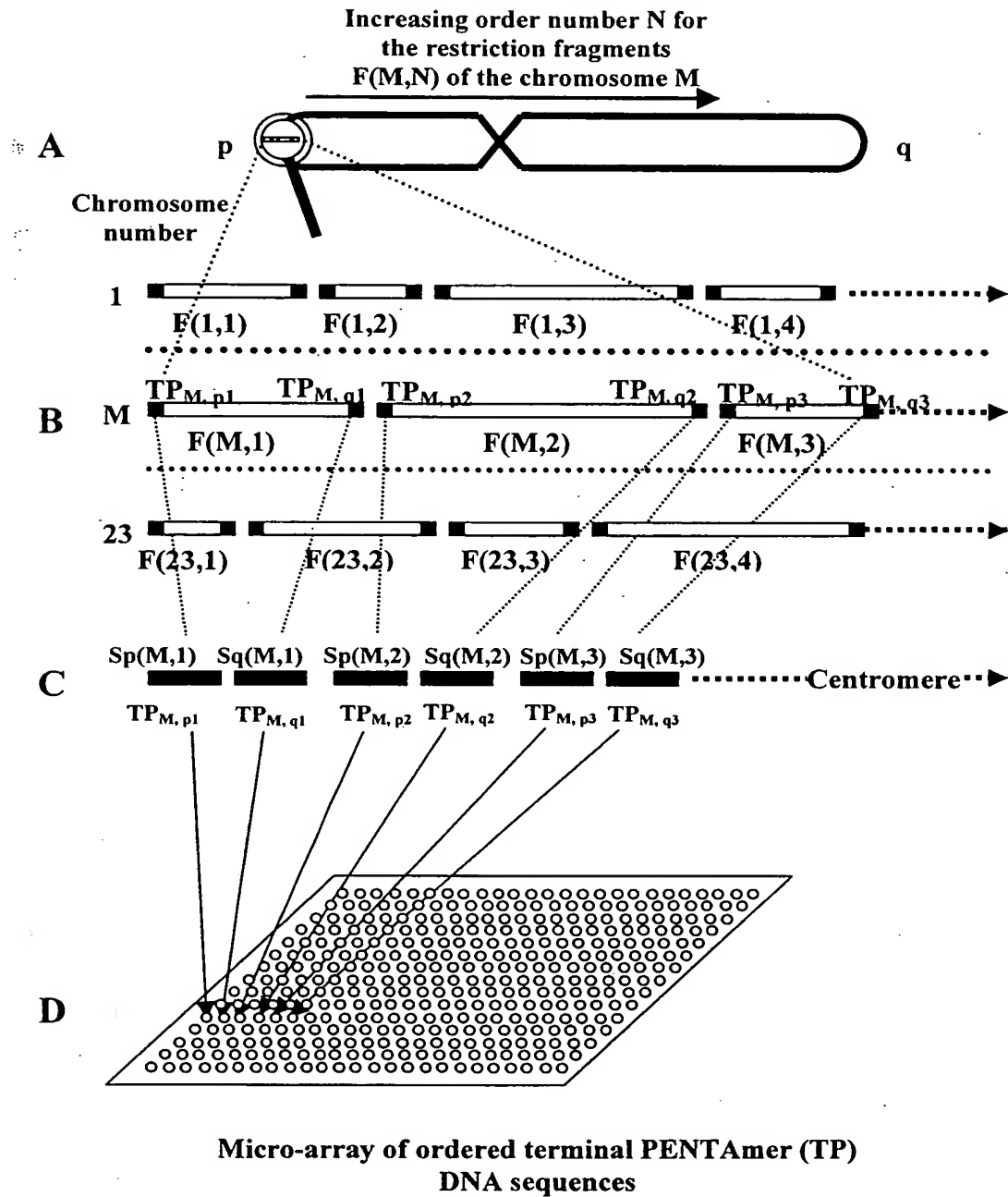


FIG. 37



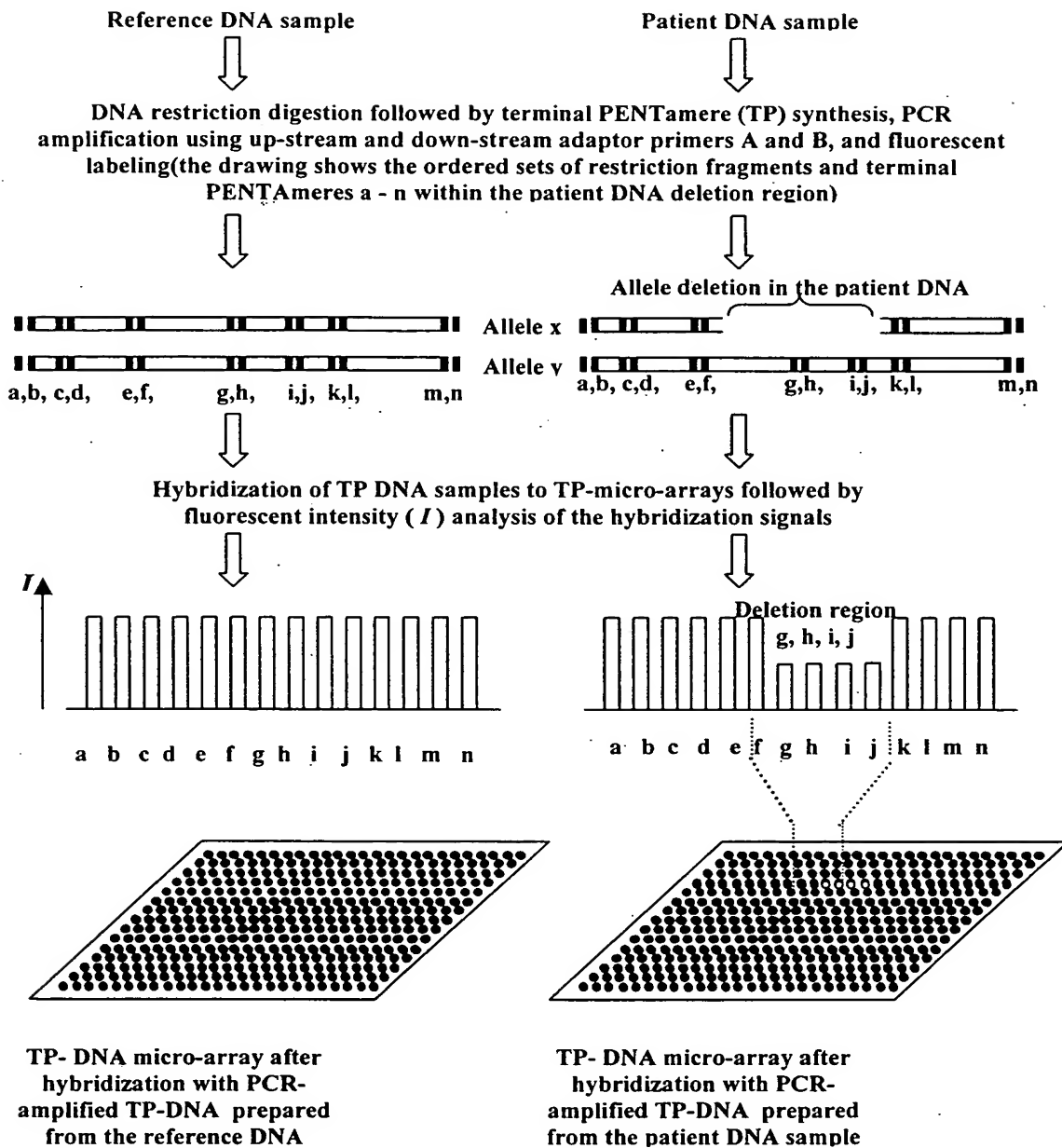


FIG. 38

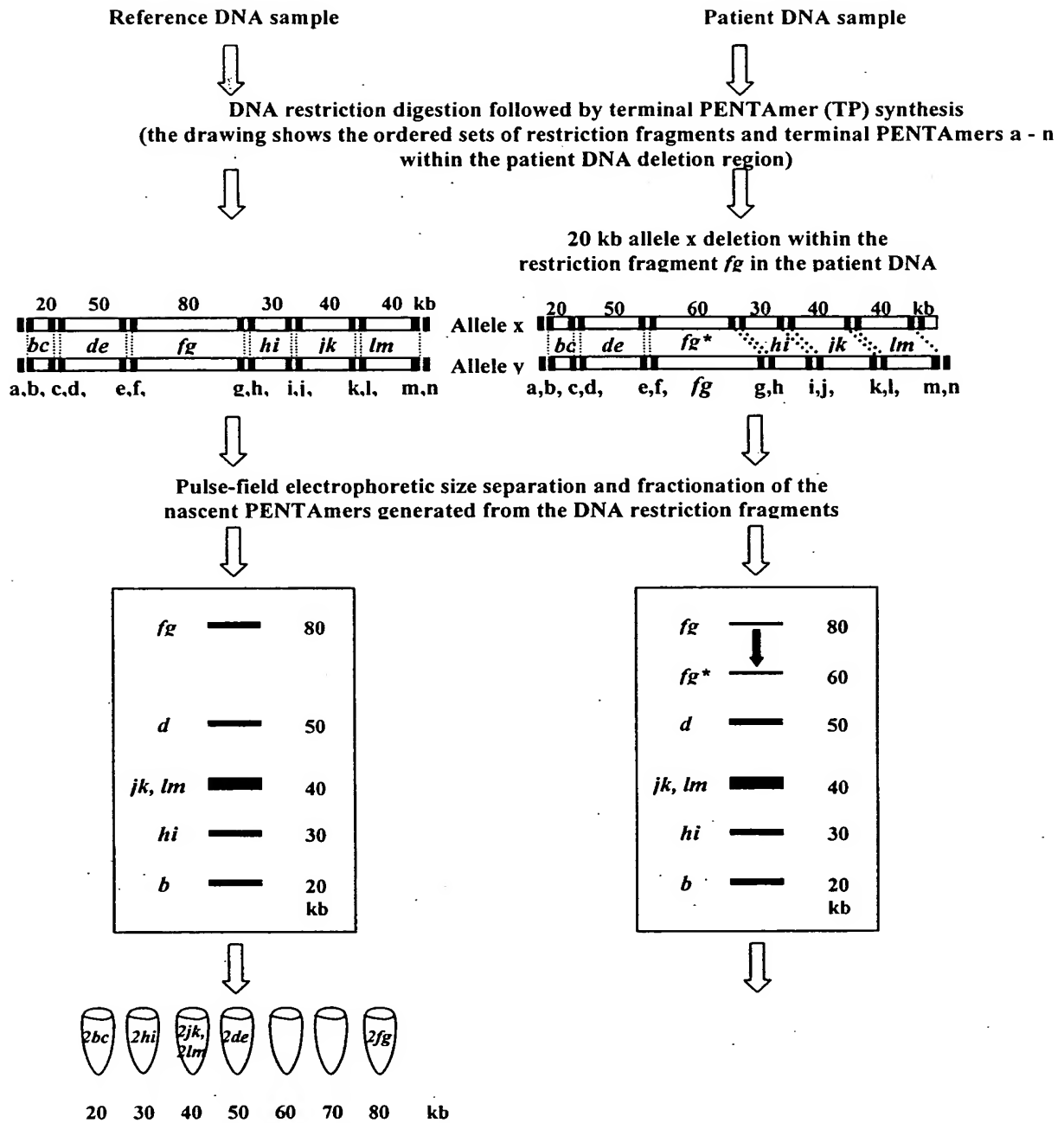
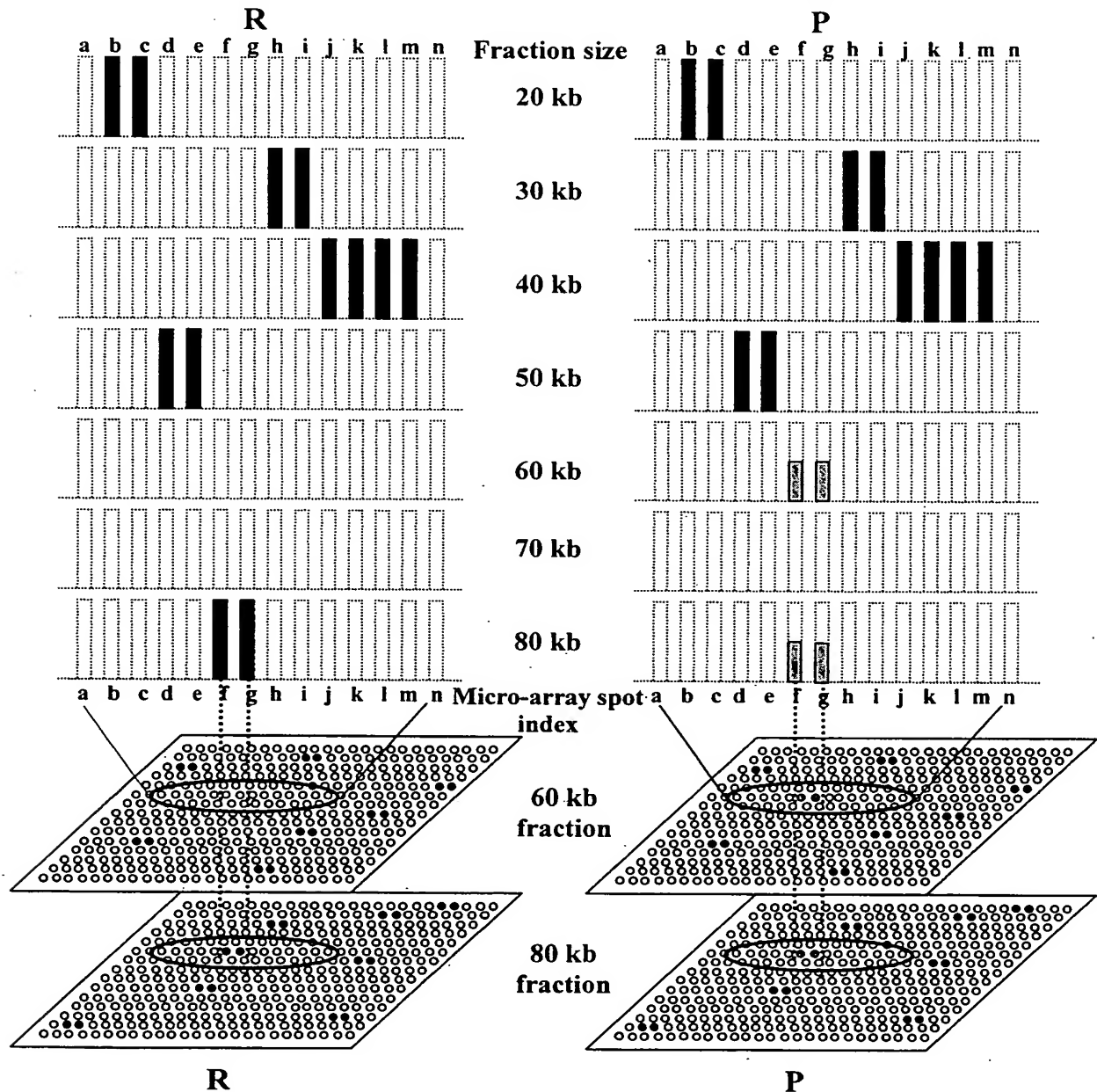


FIG. 39A

Hybridization of TP DNA size fractions to the TP-micro-array followed by  
fluorescent intensity analysis of the hybridization signals

Fluorescence intensity profiles of the TP- DNA micro-array a-n region after  
hybridization with PCR-amplified and labeled TP-DNA size fractions 20kb, 30kb, 40kb,  
50kb, 60kb, 70kb and 80kb prepared from reference (R) and patient (P) DNA samples



TP- DNA micro-array a-n region after hybridization with PCR-amplified 60kb and 80 kb  
TP-DNA size fractions prepared from the reference (R) and patient (P) DNA samples

**FIG. 39B**

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**Up-stream, terminus-attaching, nick-translation adaptor A**

5' P - GATCGCCTATACCTAGGACCATGTAA<sup>dd</sup>C 3' (SEQ ID NO. 16)  
3' <sup>dd</sup>CGGAUATGGAUCCUGGUACATTG-OH 5' (SEQ ID NO. 17)

**Acceptor-adaptor Ac**

5' - GATCGCCTATACCTAGGACCATGTAA 3' (SEQ ID NO. 18)  
3' CGGAUATGGAUCCUGGUACATTG-OH 5' (SEQ ID NO. 19)

**Recombination, nick-translation adaptor RA-(*L-cos*)**

5' P - GATCGCCTATACCTAGGACCATGTAA<sup>CGAATTC</sup>CATCA 3' (SEQ ID NO. 20)  
<sup>NH<sub>2</sub></sup>CGGAUATGGAUCCTGGUACATUGCTTAAGTAGTCCC<sup>CGC</sup>GCTGGA-OH 5' (SEQ ID NO. 21)

**Down-stream, nick-attaching adaptors B-3' (a), B-3' (b), B-3' (c) and B-3' (d)**

5'-GGGAGATCTGAATTC<sup>CCCCCCCCC</sup><sup>dd</sup>C-3' (SEQ ID NO. 22)  
3' -<sup>dd</sup>CGCCACTGGGCCCTCTAGACTTAAG - P 5' (SEQ ID NO. 23) (a)

5'-GTTACATGGTCCTAGGTATAGGC GCGGTGACCCGGGAGATCTG<sup>CCCCCCCCC</sup>-3' (SEQ ID NO. 24)  
3'- AATGTACCAGGATCCATATCCGCGCCACTGGGCCCTCTAGAC - P 5' (SEQ ID NO. 25) (b)

5'-GGGAGATTCTGAATTC<sup>AAAAAAAAA</sup><sup>dd</sup>A-3' (SEQ ID NO. 26)  
3' -<sup>dd</sup>AGCCACTGGGCCCTCTAGACTTAAG - P 5' (SEQ ID NO. 27) (c)

5'-GTTACATGGTCCTAGGTATAGGC GCGGTGACCCGGGAGATCTG<sup>AAAAAAAAA</sup>-3' (SEQ ID NO. 28)  
3- AATGTACCAGGATCCATATCCGCGCCACTGGGCCCTCTAGAC - P 5' (SEQ ID NO. 29) (d)

**Oligo-construct with nick**

5'-Biotin-GCGGTGACCCGGGAGATCTGAATTCA GGGCGGCGACCT-3' (SEQ ID NO. 30 & 31)  
3'- CGCCACTGGGCCCTCTAGACTTAAGTCCC<sup>CGC</sup>GCTGGA - P-5' (SEQ ID NO. 32)

32P-A      nick  
          ↓      ↙

a) for a nomenclature of the adaptors A and B-3' see section 6

**Fig. 40**

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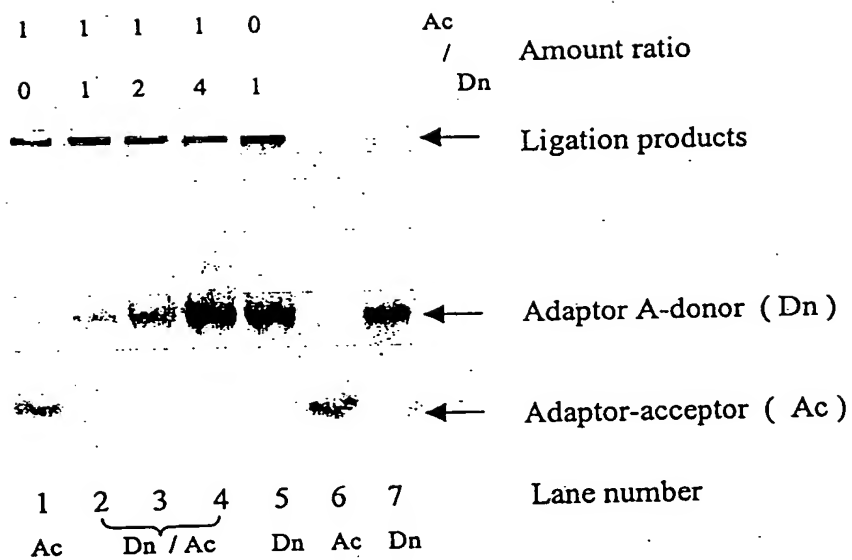


Fig. 41

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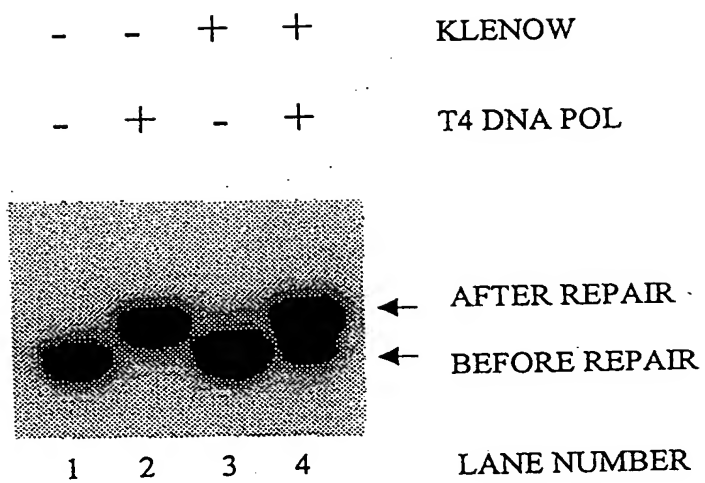


Fig. 42

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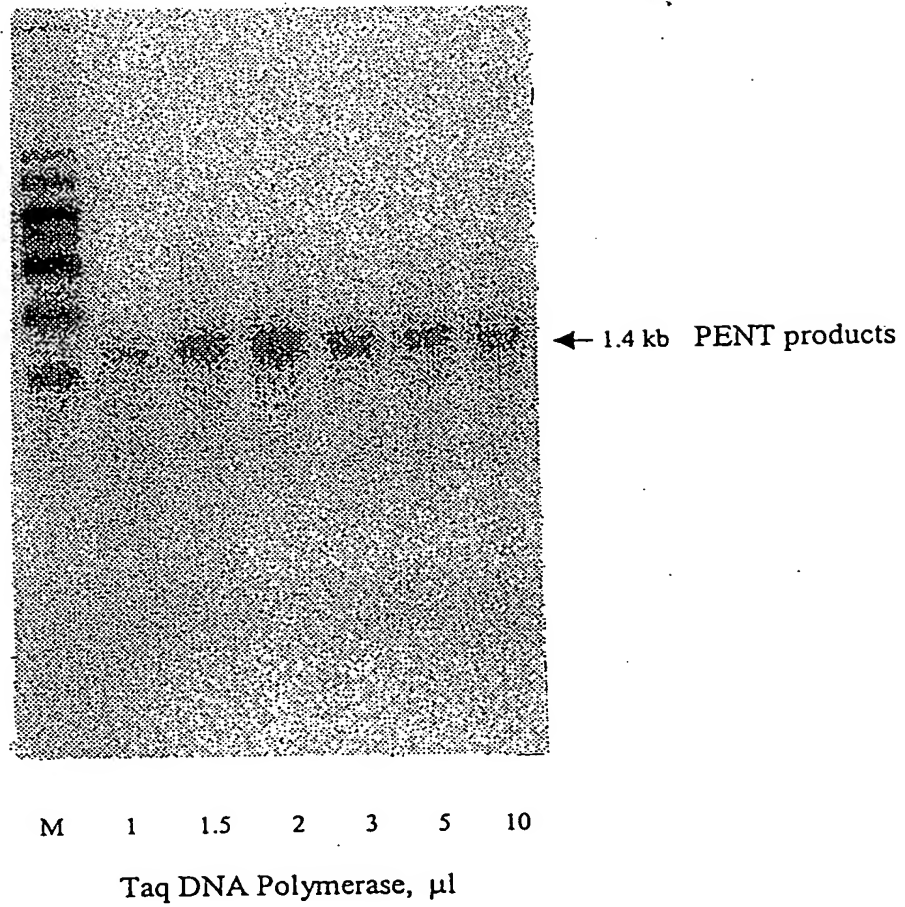


Fig. 43

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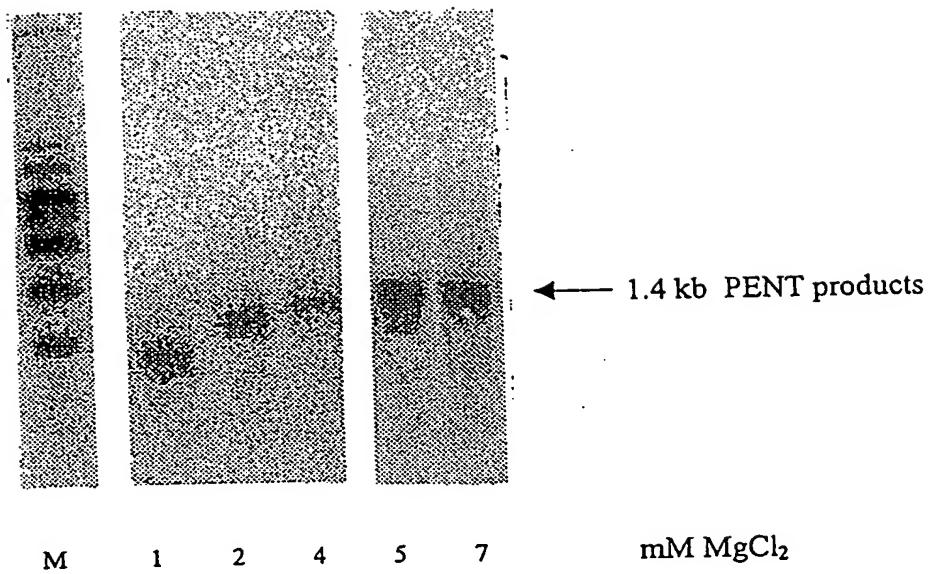


Fig. 44



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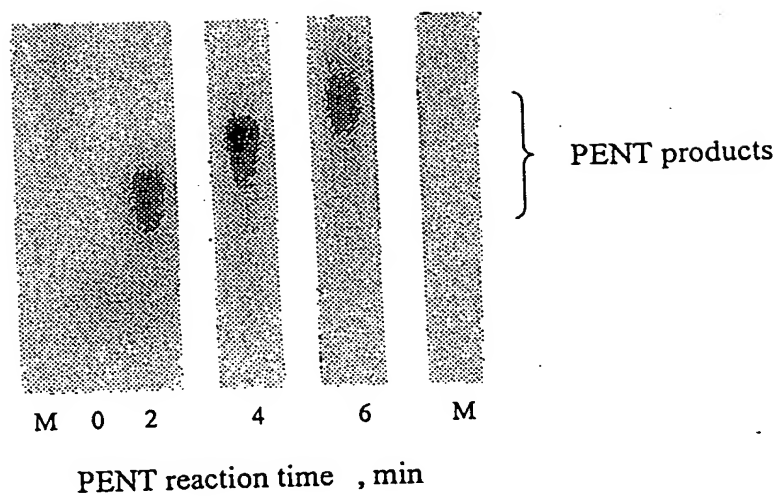


Fig. 45

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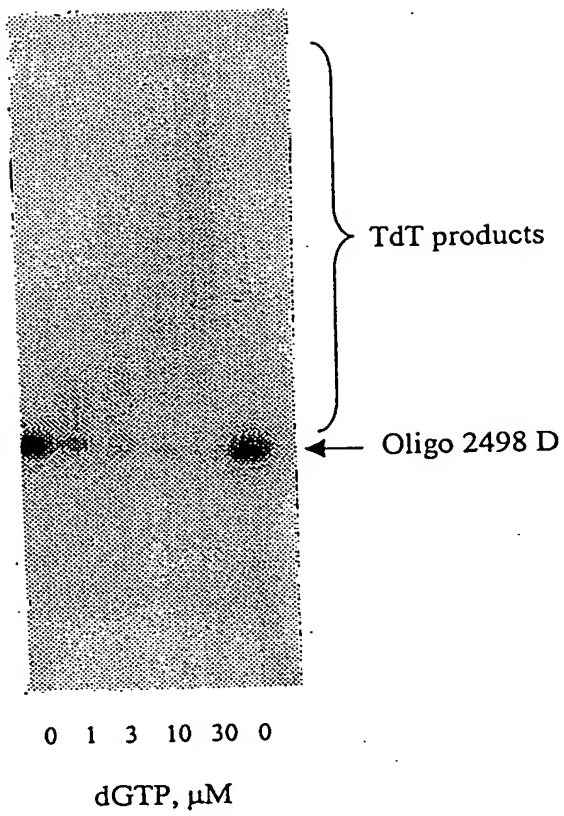


Fig. 46

M	EtOH	Ph/Cl	Microcon 100
---	------	-------	--------------

M

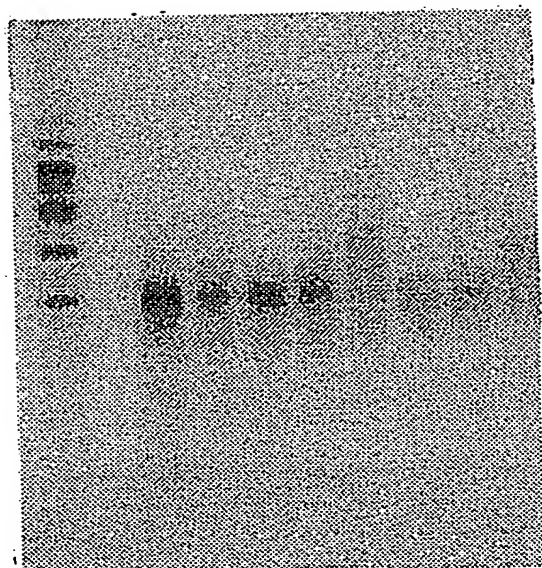
EtOH

Ph/Cl

## Microcon 100

$$+ \quad - \quad + \quad - \quad + \quad - \quad - \quad +$$

TdT



$\underbrace{\hspace{1.5cm}} \quad \underbrace{\hspace{1.5cm}} \quad \underbrace{\hspace{1.5cm}} \quad \underbrace{\hspace{1.5cm}}$

Tube number

Fig. 47

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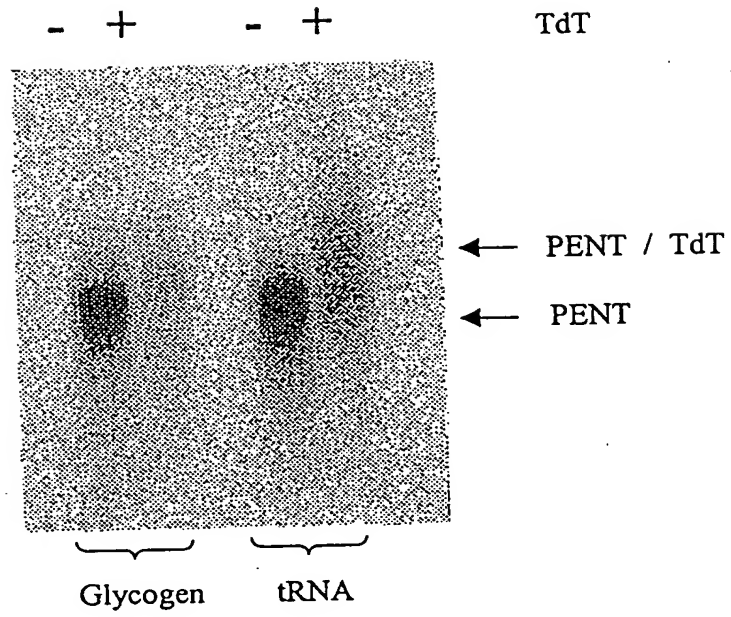


Fig. 48

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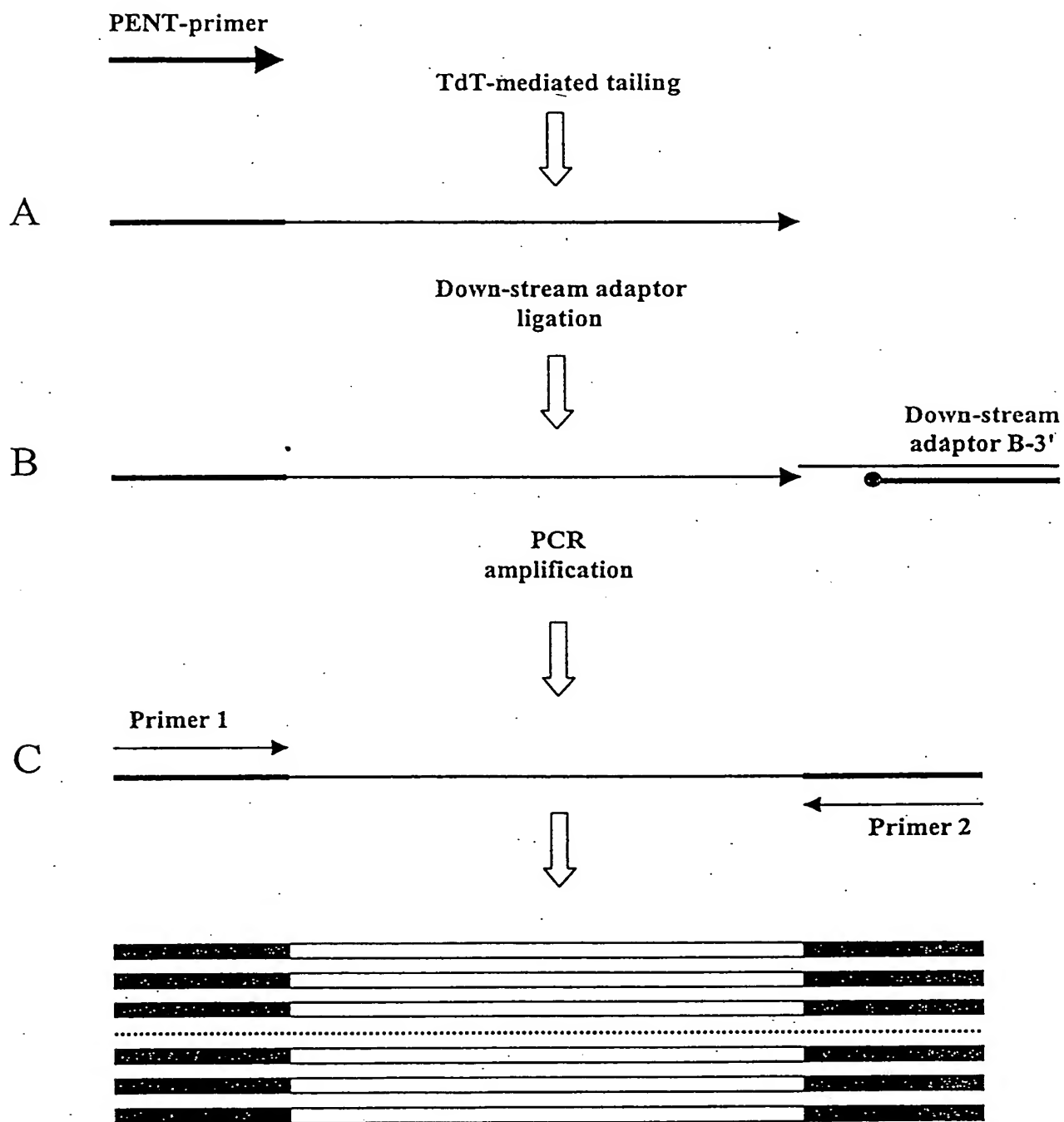


Fig. 49

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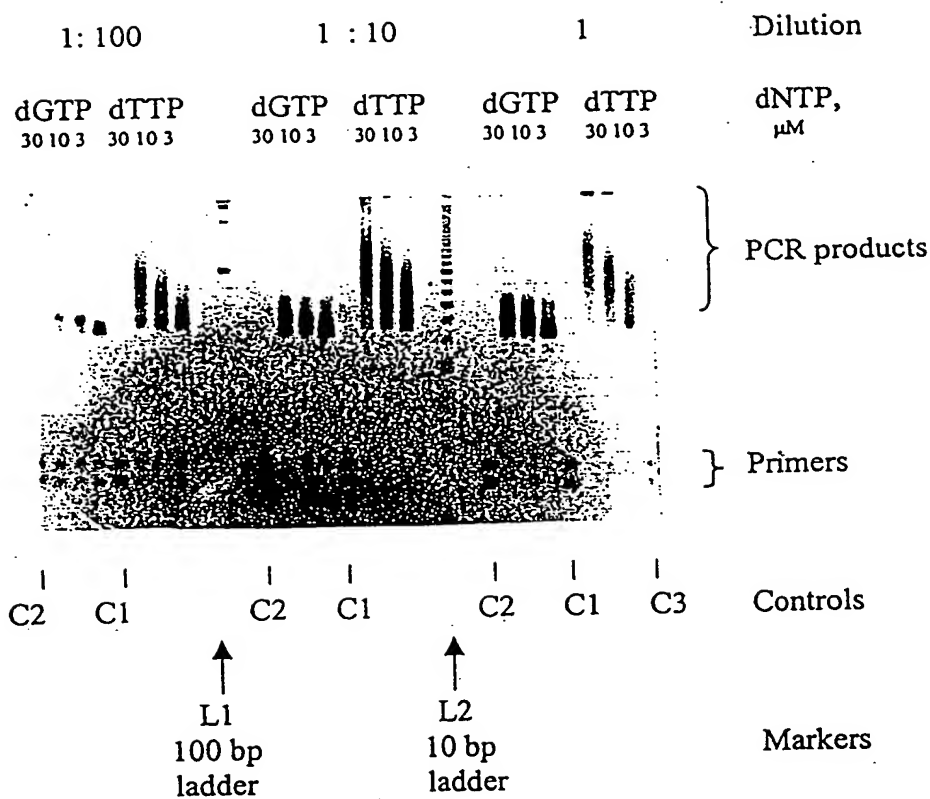


Fig. 50

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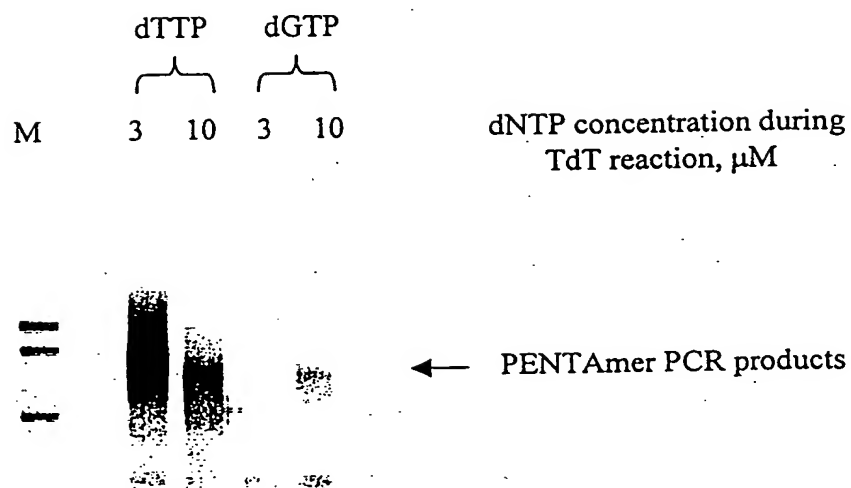


Fig. 51

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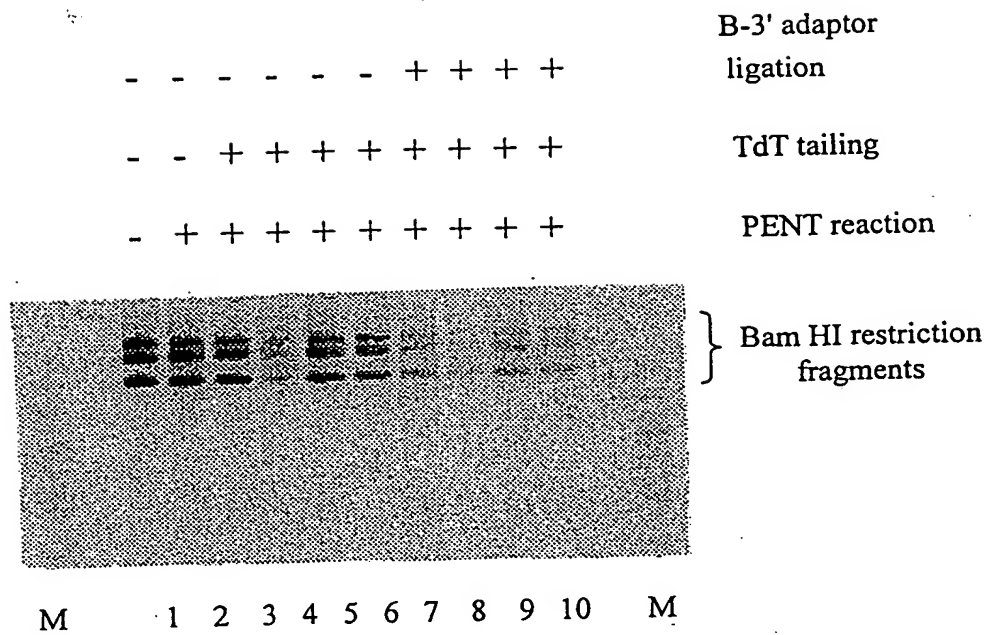


Fig. 52



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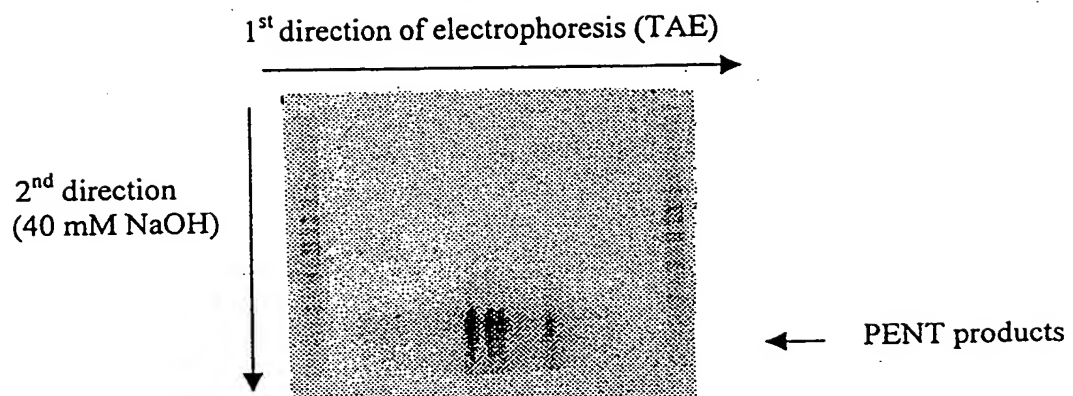


Fig. 53

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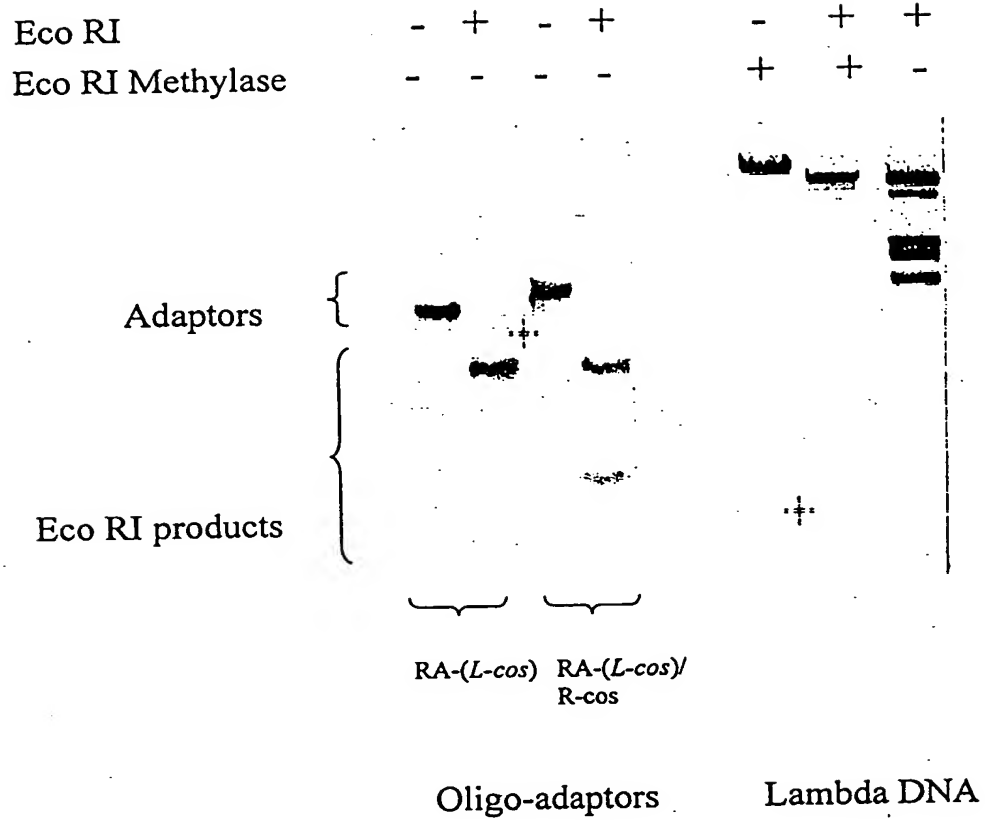


Fig. 54

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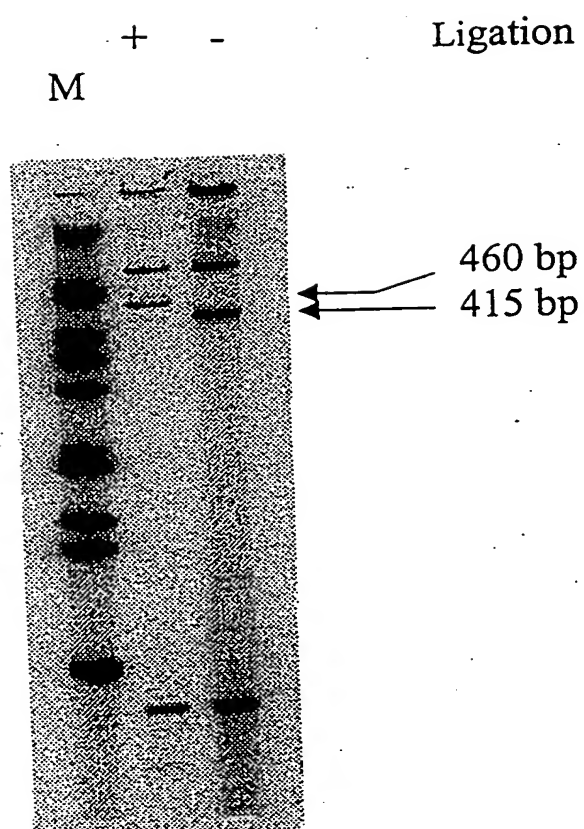


Fig. 55

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Lambda DNA      Human leukocyte  
DNA

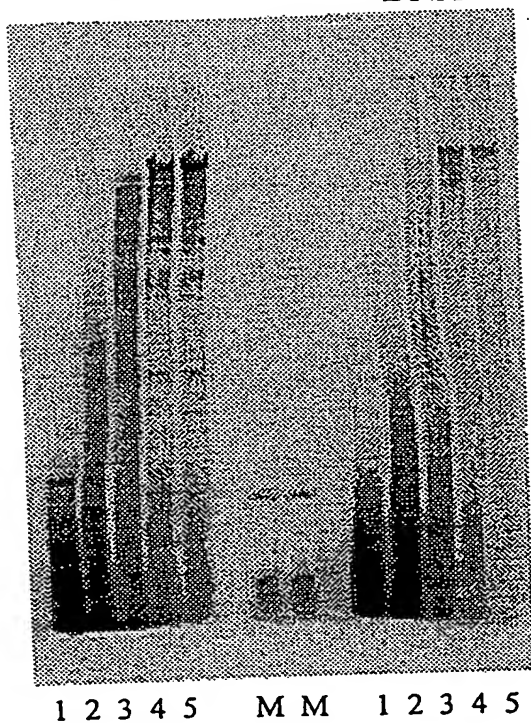
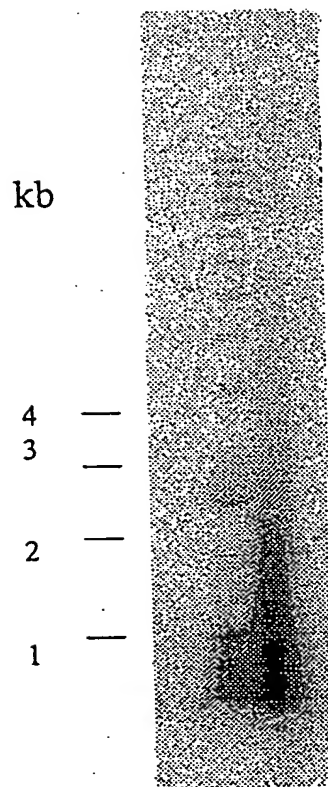


Fig. 56

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M DNA

Fig. 57

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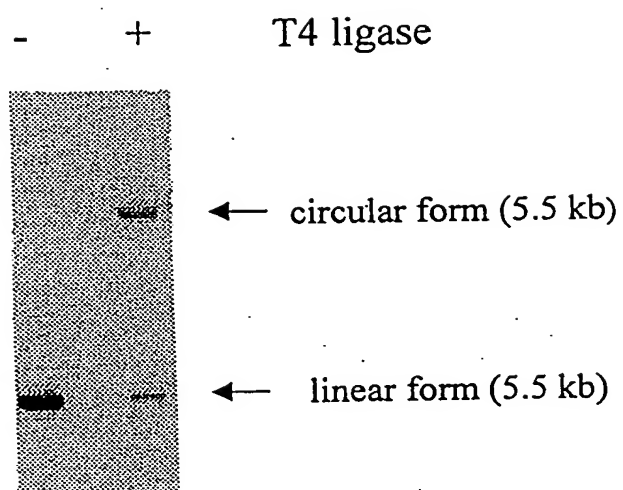


Fig. 58

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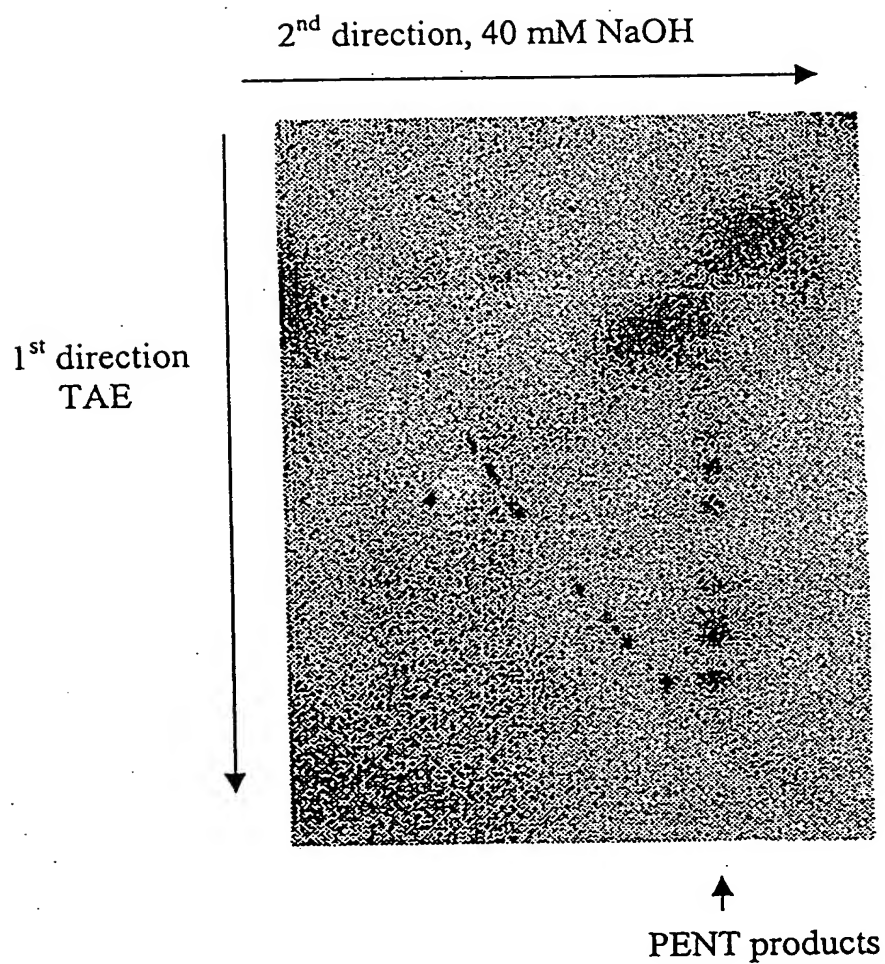


Fig. 59

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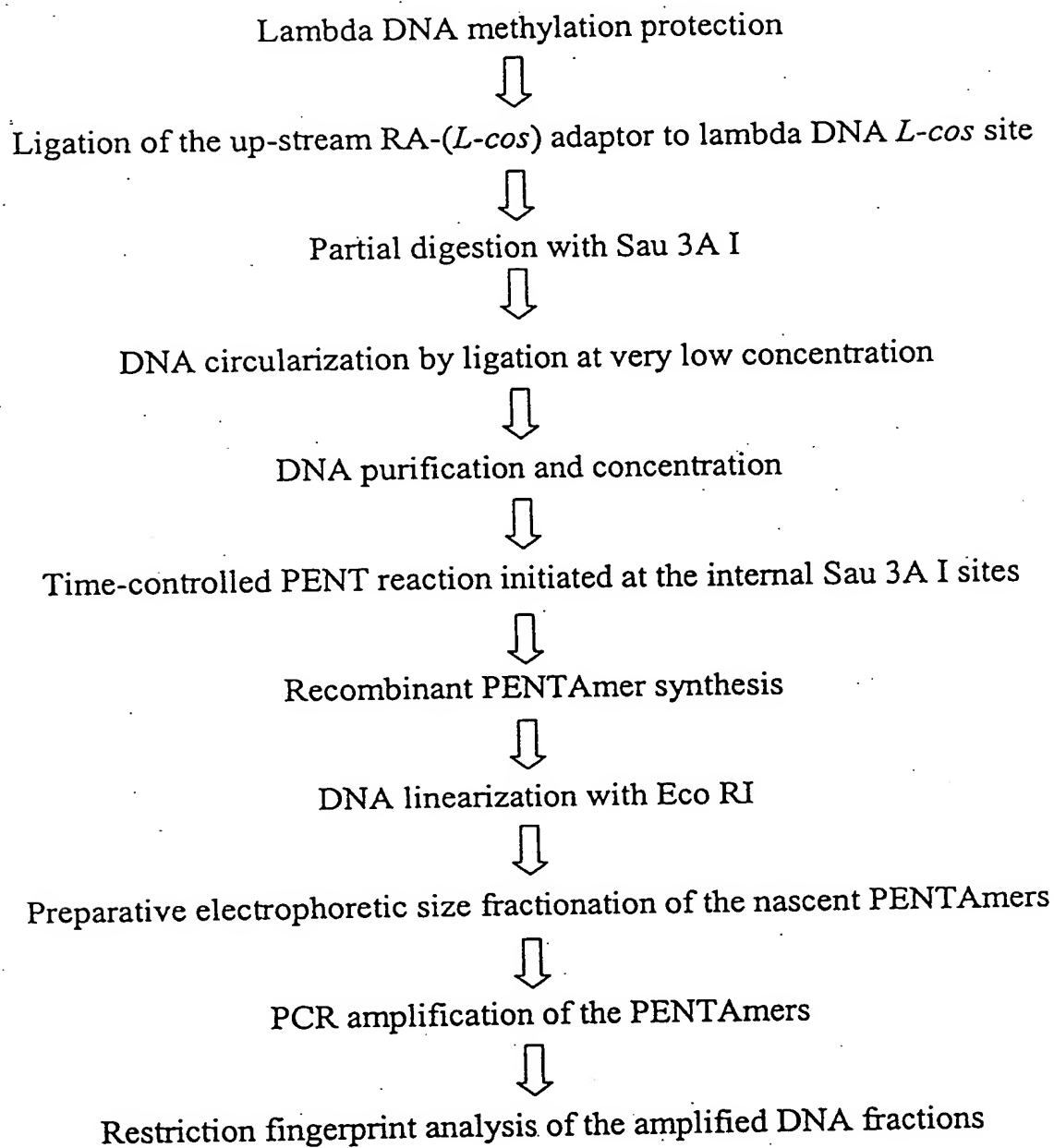
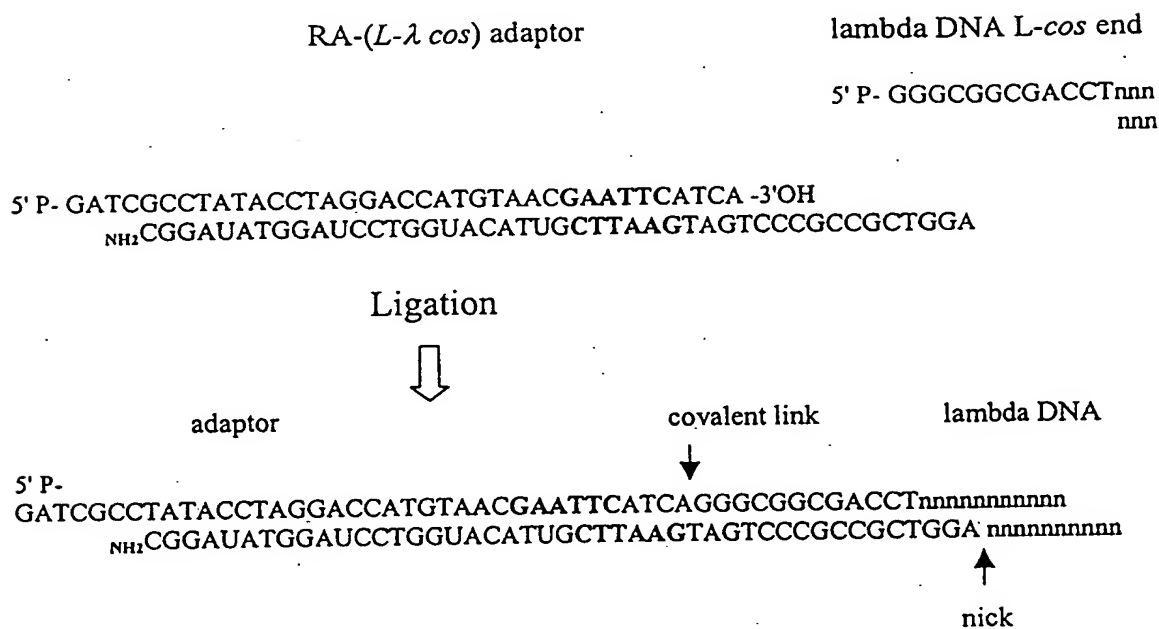


Fig. 60

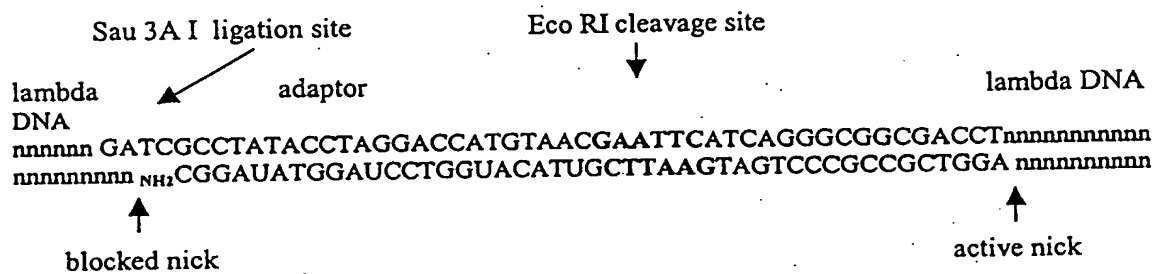


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# A The RA-( $L$ - $\lambda$ *cos*) adaptor - Lambda DNA junction structure



# B The recombinant junction formed by a circularization reaction .



# C The 5' -end of the PENTAmer

nnnnnnnnnnctagCGGAUATGGAUCCTGGUACATUGCTTAA - 5'

Fig. 61

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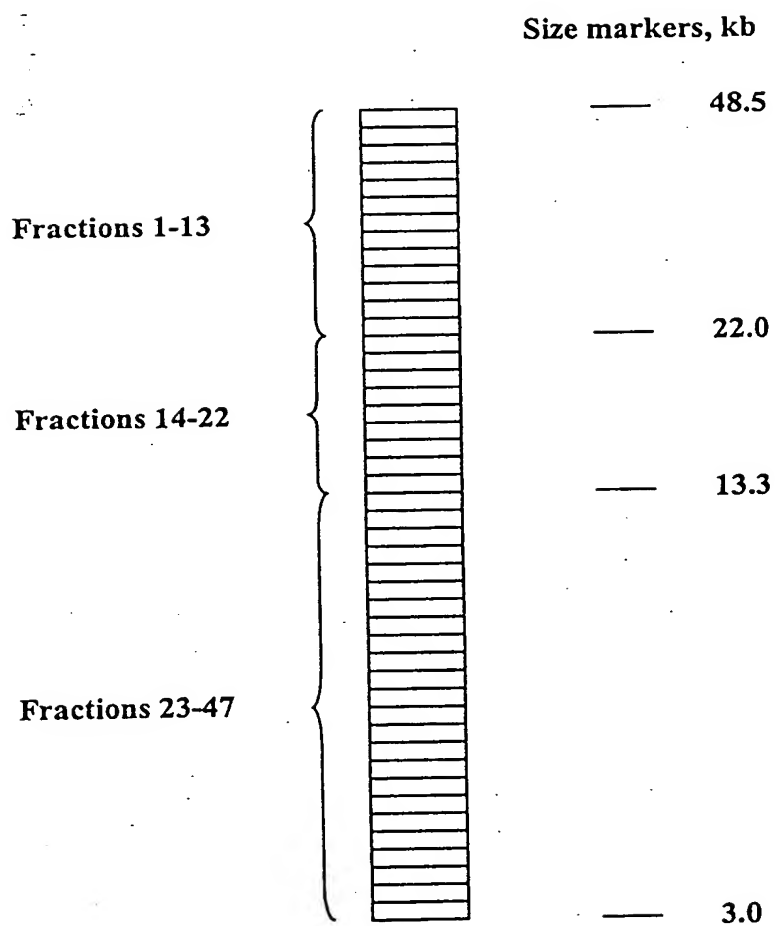


Fig. 62

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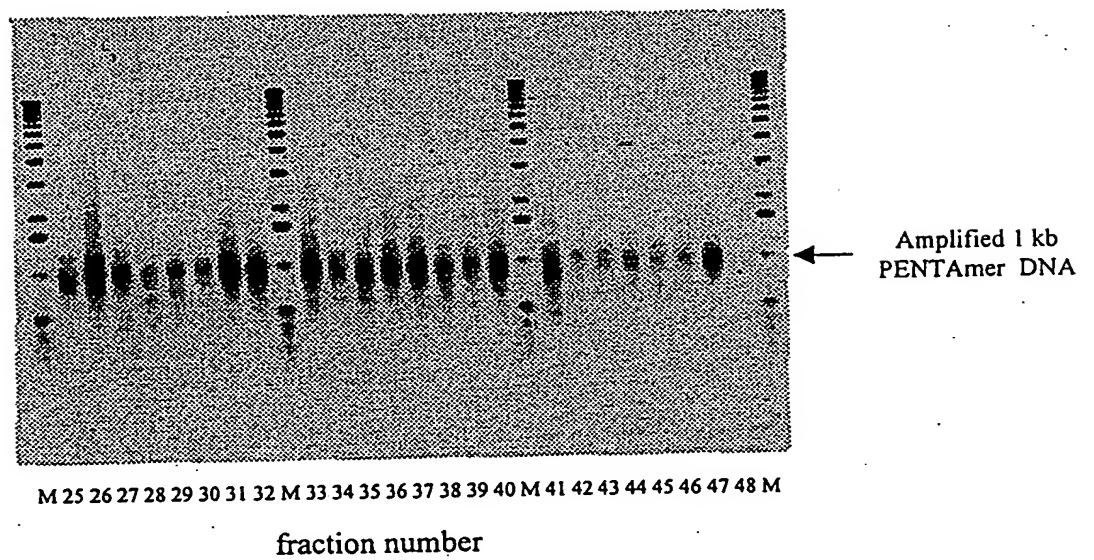
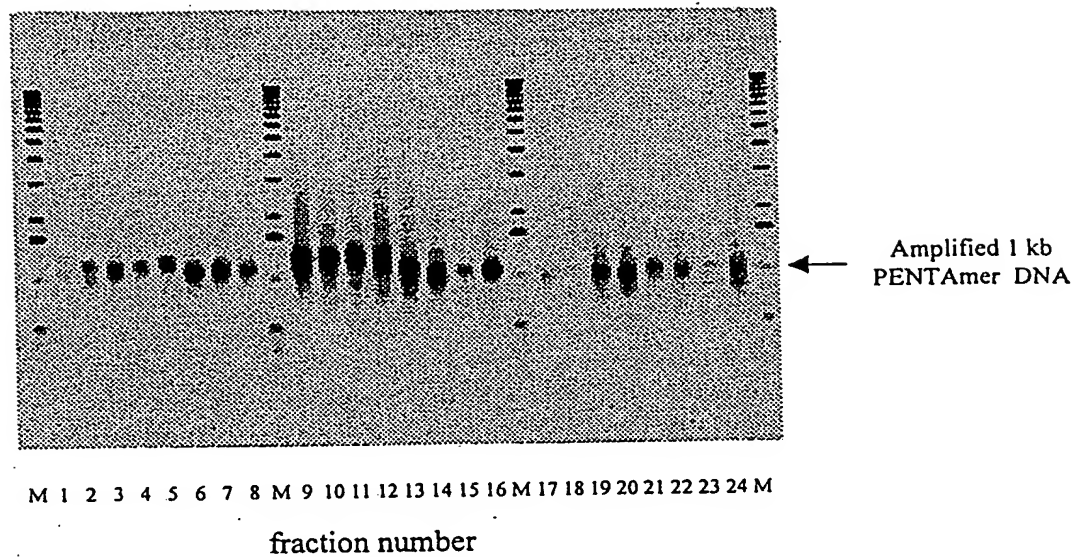
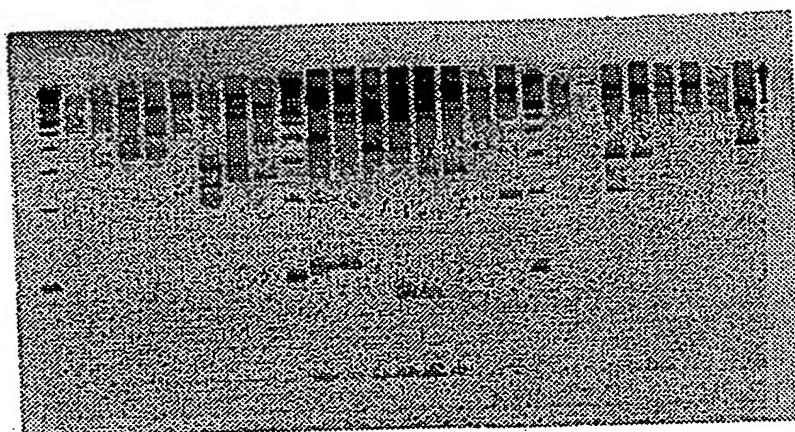


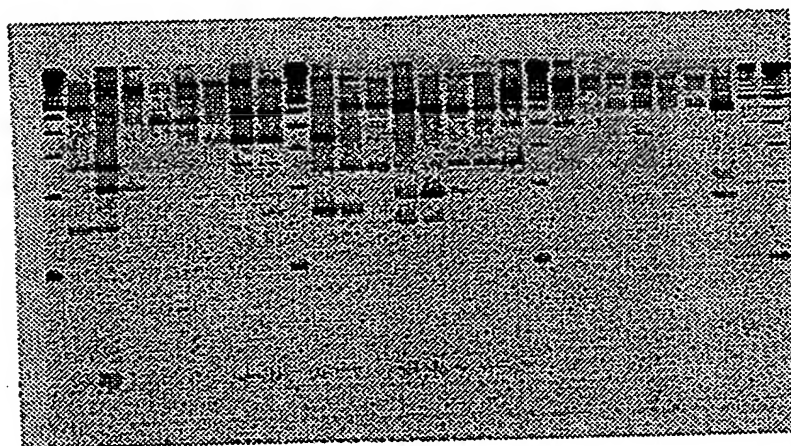
Fig. 63

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M 1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16 M 17 18 19 20 21 22 23 24 M

fraction number

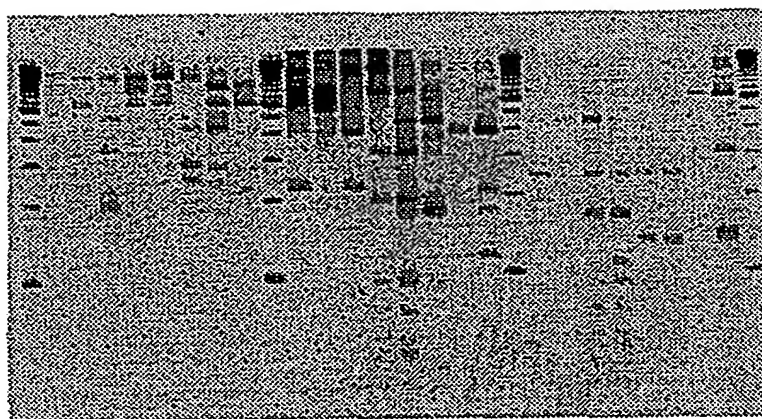


M 25 26 27 28 29 30 31 32 M 33 34 35 36 37 38 39 40 M 41 42 43 44 45 46 47 48 M

fraction number

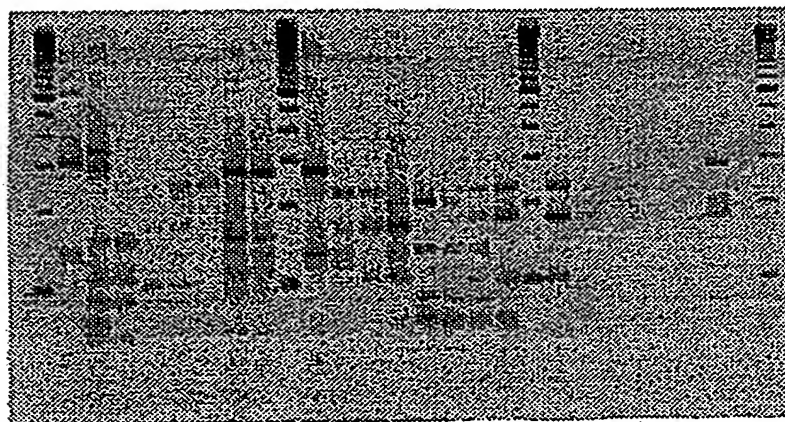
Fig. 64

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M 1 2 3 4 5 6 7 8 M 9 10 11 12 13 14 15 16 M 17 18 19 20 21 22 23 24 M

fraction number



M 25 26 27 28 29 30 31 32 M 33 34 35 36 37 38 39 40 M 41 42 43 44 45 46 47 48 M

fraction number

Fig. 65

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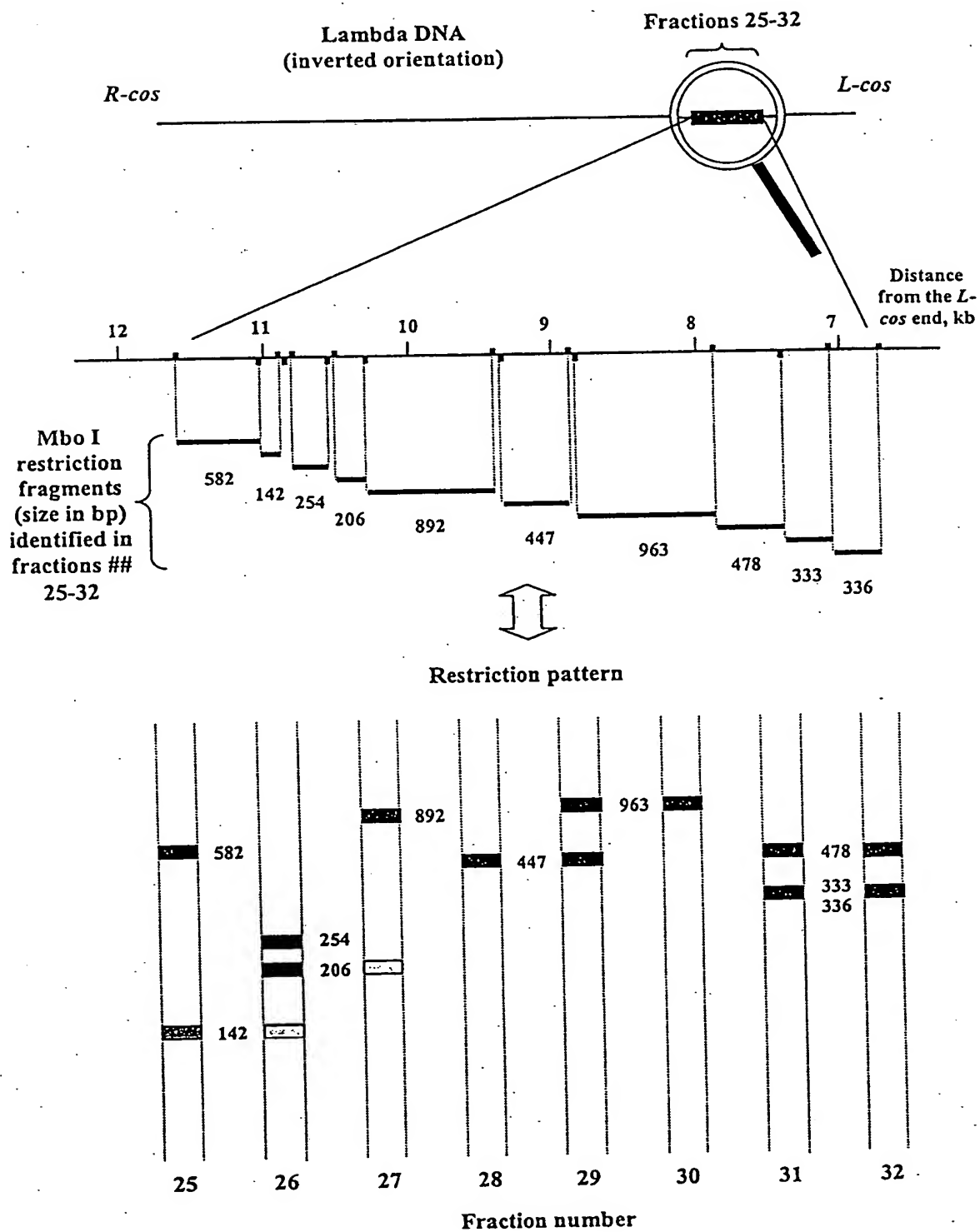


Fig. 66

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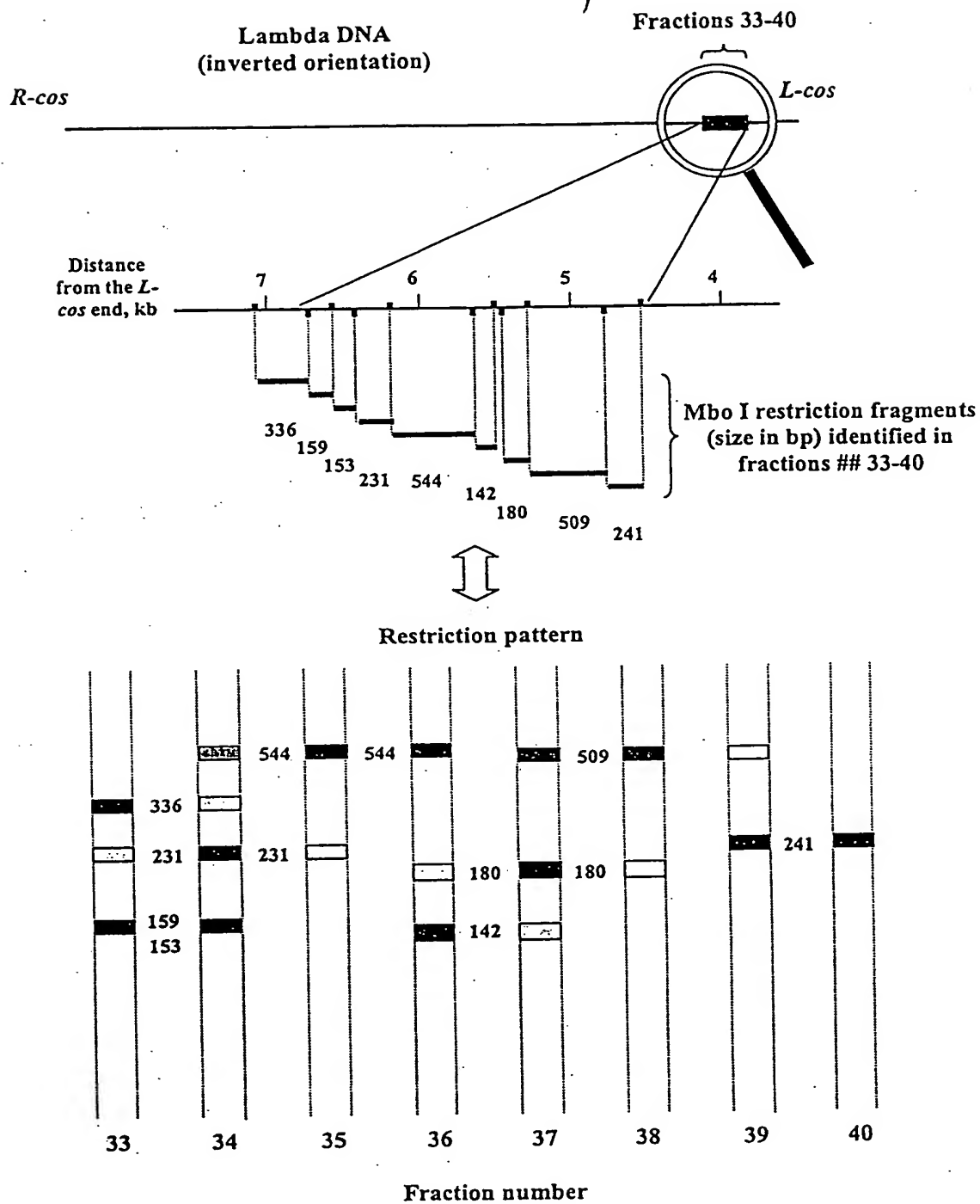


Fig. 67

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FIG. 68



- A) RAL/RA2 (Methylation Dpn-I Activation)
- RA1 5'-GATCTGAGGTTGTAGAAGACTCGGACGATACACATGCACCGTCGGTGCAGTCGTAATCCAGTCCCGA (SEQ ID NO:69) (SEQ ID NO:70 below)  
 AGCCACGTCAGCATTAGTCAGGGCTCCTGTGCGGATAAAACGATCGGGGACTCGTTATTGATCGCTAG-5' RA2
- Assembled RA1 5'-(P)- GATCTGAGGTTGTAGAAGACTCGGACGATACACATGCACCGTCGGTGCAGTCGTAATCCAGTCCCGATCTCAGAGCGTT\  
 B3'-ACTCCAAACATCTTCTGAGCGCTGTATGTACGTGGCAGCCACTGCAGCATTAGTCAGGGCTAGAGTCTCGCTT/ (SEQ ID NO:71)
- RA1 Component Oligos:
- RA1(A) 5'-(P)- GATCTGAGGTTGTAGAAGACTCGGACGATACACATGCACCGTCGGTGCAGTCGTAATCCAGTCCCGATCTC-3' (SEQ ID NO:72)  
 RA1(B) 5'-CTTCTACAACTCA-B3' (SEQ ID NO:73)  
 RA1(C) 5'-(P)-CGGTGCATGTGTATCGTCCGAGT-3' (SEQ ID NO:74)  
 RA1(D) 5'-(P)-AGAGCGTTTTTCGCTCTGAGATCGGACTGGATTACGACTGCACCGA-B3' (SEQ ID NO:75)
- Assembled RA2 5'-(P)- GATCGCTAGTTATTGCTCACGGGCTAGCAAAATAGCGGTGCTCTCGGACTGGATTACGACTGCACCGATCTCAGCGG-T-T\  
 B3'-CGATCAATAACGAGTCCCGATCGTTTTATCGGCACAGAGCCCTGACCTAATGCTGACGTGGCTAGAGTCTCGC-T-T/ (SEQ ID NO:76)
- RA2 Component Oligos:
- RA2(A) 5'-(P)-GATCGCTAGTTATTGCTCACGGGCTAGCAAAATAGCGGTGCTCTCGGACTGGATTACGACTGCACCGATCTC-3' (SEQ ID NO:77)  
 RA2(B) 5'-GAGCAATAGTGC-B3' (SEQ ID NO:78)  
 RA2(C) 5'-(P)-GGACAGCGCTATTTTGTAGCCCGT-3' (SEQ ID NO:79)  
 RA2(D) 5'-(P)-AGAGCGTTTTTCGCTCTGAGATCGGTGCAGTCGTAATCCAGTCCCGA-B3' (SEQ ID NO:80)
- B) Simplified Recombinant Adapters Sra1/Sra2
- Sra 1 5'-GATCTGAGGTTGTGAAGACTCGGACGATACACAGCTGGGTTGAGGAAGTCGTAAATA  
 TGTGCCACCCAACTCCTTCAGCATTTATTATTGGTAGGGTTGCTTATTGATCGCTAG-5' P Sra 2
- Sra 1A 5'-P-GATCTGAGGTTGTGAAGACTCGGACGATACACAGCTGGGTTGAGGAAGTCGTAAATA-3' (SEQ ID NO:81)  
 Sra 1B B3'-ACTCCAACTTC-5' (SEQ ID NO:82)  
 Sra 1C B3'-ACTCCAACTTCGTAGCCTGCT-5' (SEQ ID NO:83)  
 Sra 1D B3'-TGTGCCACCCAACTCCTTCAGCATTTATTATTGGTAGGGTTGCTTATTGATCGCTAG-5' P Sra 2A (SEQ ID NO:85)  
 5'-CAGCAATACTAGC-B3' Sra 2B (SEQ ID NO:86)  
 5'-HACCATCCCAACAGCAATACTAGC-B3' Sra 2C (SEQ ID NO:87)  
 5'-ACACGCTGGTTGAGGAAGTCGTAAATA-B3' Sra 2D (SEQ ID NO:88)
- C) Sra1' Expanded complementarity with original Sra2
- 5'-P-GATCTGAGGTTGTGAAGACACGCTGGGTTGAGGAAGTCGTAAATAAACCATCCAA-3' Sra 1A' (SEQ ID NO:89)  
 B3'-ACTCCAACTTC-5' Sra 1B (SEQ ID NO:82)  
 B3'-TGTGCCACCCAACTCCTTCAGCATTTATTATTGGTAGGGTTGCTTATTGATCGCTAG-P5' Sra 1D (SEQ ID NO:84)  
 B3'-TTATTGGTAGGGTT-5' Sra 1H (SEQ ID NO:90)

FIG. 69

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### Simplified recombinant adapter (Sra) oligos

Sra 1A P5'-GATCTGAGGTTGTTGAAGACTCGGACGATACACACGCTGGGTTGAGGAAGTCGTAAATA-3' (SEQ ID NO:91)

Sra 1B 5'-CTTCAACAACCTCA-B3' (SEQ ID NO:92)

Sra 1C 5'-TCGTCCGAGTCTTCAACAACCTCA-B3' (SEQ ID NO:93)

Sra 1D 5'-TATTTACGACTTCCTCAACCCAGCGTGT-B3' (SEQ ID NO:94)

Sra 2A P5'-GATCGCTAGTTATTGCTGTTGGGATGGTTATTATTACGACTTCCTCAACCCAGCGTGT-3' (SEQ ID NO:95)

Sra 2B 5'-CAGCAATAACTAGC-B3' (SEQ ID NO:96)

Sra 2C 5'-AACCATCCCAACAGCAATAACTAGC-B3' (SEQ ID NO:97)

Sra 2D 5'-ACACGCTGGGTTGAGGAAGTCGTAAATA-B3' (SEQ ID NO:98)

Sra 1A' P5'-GATCTGAGGTTGTTGAAGACACGCTGGGTTGAGGAAGTCGTAAATAAATAACCATCCCAA-3' (SEQ ID NO:99)

Sra 1H 5'-TTGGGATGGTTATT-B3' (SEQ ID NO:100)

### Lambda recombination screening oligos

Total(+) 5'-AGGTTGTAGAAGACTCGG-3' (SEQ ID NO:101)

Total(-) 5'-GCTAGTTATTGCTCACGG-3' (SEQ ID NO:102)

Intra(+34273) 5'-GCATCGCTTGAATTGTCC-3' (SEQ ID NO:103)

Intra(-28119) 5'-TGCTCTCGGAATATCAAT-3' (SEQ ID NO:104)

Inter(+34273) 5'-GCATCGCTTGAATTGTCC-3' (SEQ ID NO:105)

Inter(-34599) 5'-ATATTCAGGCCAGTTATC-3' (SEQ ID NO:106)

### E-coli recombination screening oligos

B1(RP) 5'-CTTACACCGGCAAGTGAAAG-3' (SEQ ID NO:107)

B1(PCR) 5'-CGCTGCCGGAGCTGTTAGACAATTC-3' (SEQ ID NO:108)

B1(NEST) 5'-GCCTGCAAGCCGGTGTAGACATCAC-3' (SEQ ID NO:109)

B3(RP) 5'-CTGCAGGCCAGCGAGACAGAT-3' (SEQ ID NO:110)

B3(PCR) 5'-GTTGTGGCCTTCCAGTAAGGTCC-3' (SEQ ID NO:111)

B3(NEST) 5'-GCAAAATAGCTGGCTGGCAGGTGTAGG-3' (SEQ ID NO:112)

B5(RP) 5'-TAGGGCGGCATCAGGTAATAC-3' (SEQ ID NO:113)

B5(PCR) 5'-TGCCGCCGTTTCGCATCCATACCA-3' (SEQ ID NO:114)

B5(NEST) 5'-TTCCCTGCCTGGTCGCCGTATCTGTG-3' (SEQ ID NO:115)

B8(RP) 5'-TGAAGGATACGGAAGCAGAAA-3' (SEQ ID NO:116)

B8(PCR) 5'-GCCATTGCTGATTGCCACCCAGACAA-3' (SEQ ID NO:117)

B8(NEST) 5'-CTCTATCGCTCGGCCTAAGTCTTTAC-3' (SEQ ID NO:118)

B12(RP) 5'-GCGGTCGGCGTGGATAAAGTA-3' (SEQ ID NO:119)

B12(PCR) 5'-GTGAGCGGGATGAACGAACCTTA-3' (SEQ ID NO:120)

B12(NEST) 5'-CTGCGCCAGGGCTTCCAGACATTGTG-3' (SEQ ID NO:121)

**FIG. 70**

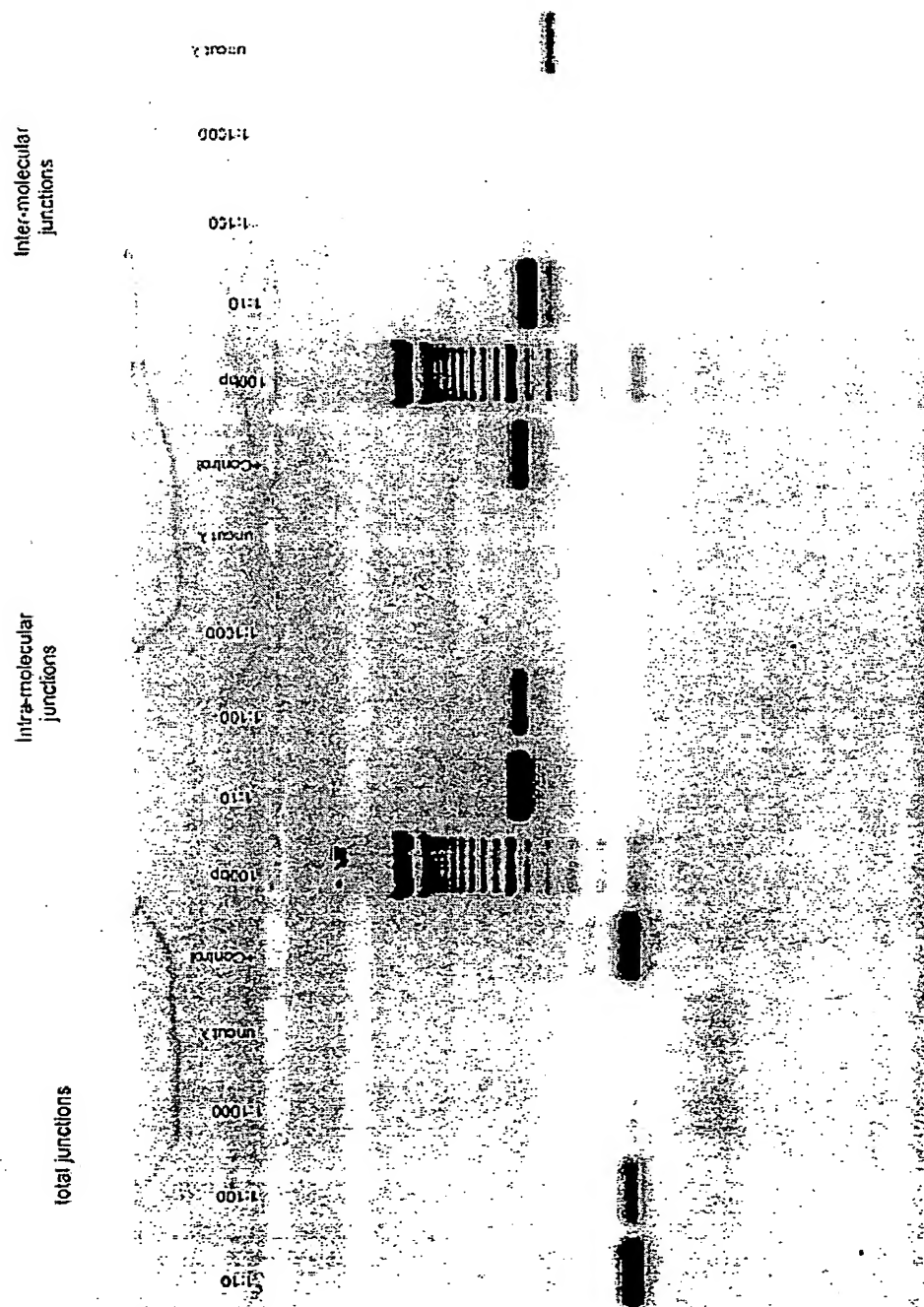
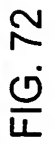


FIG. 71



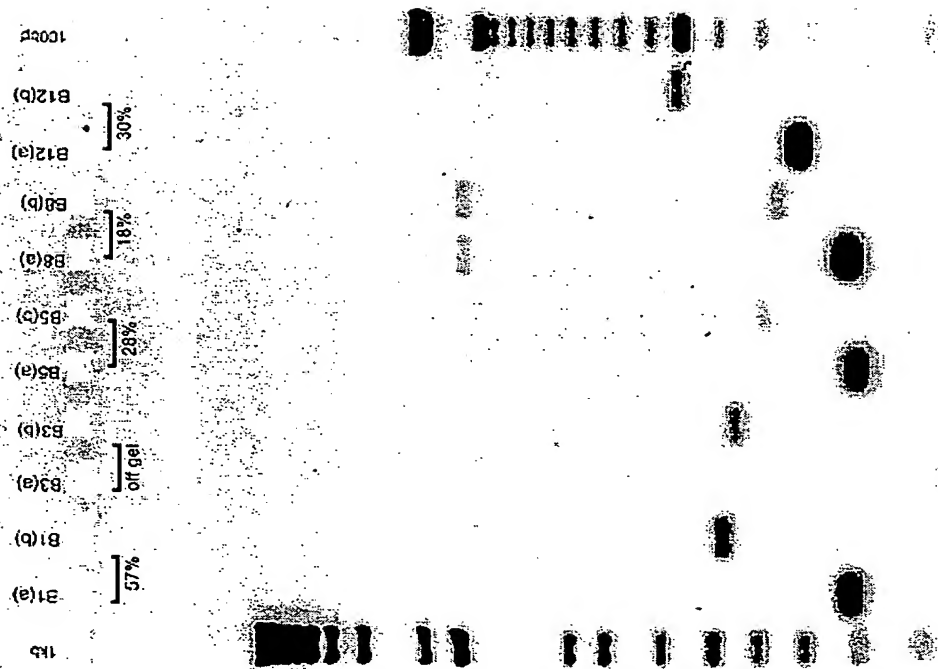


FIG. 73

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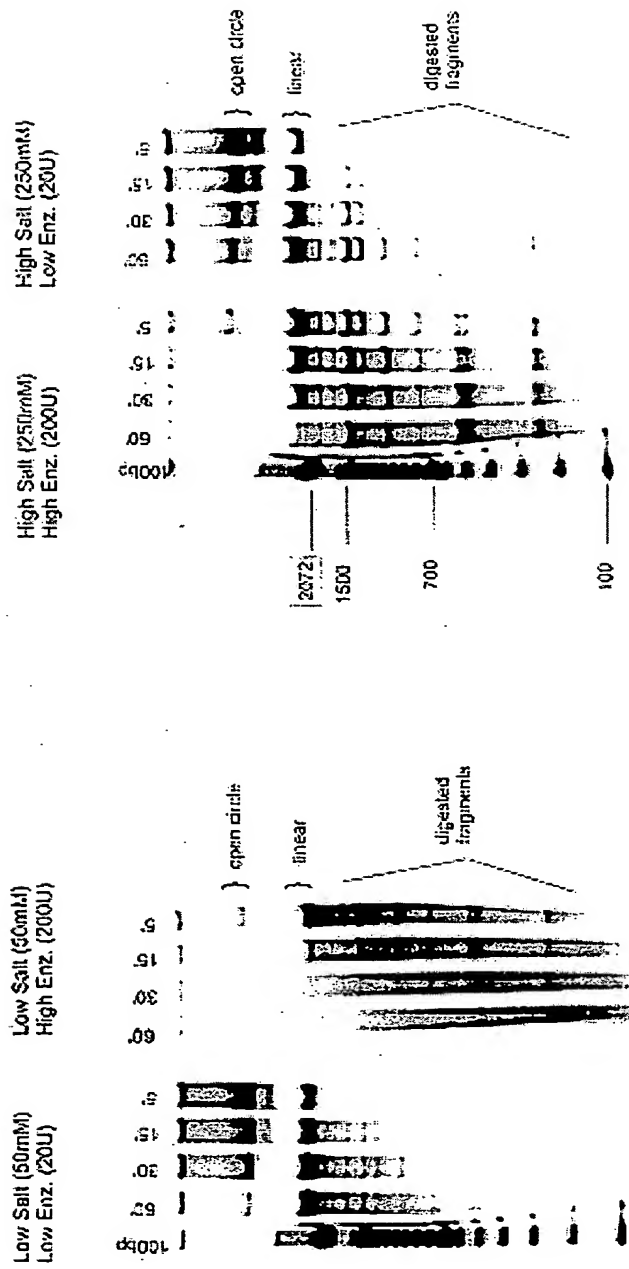


FIG. 74

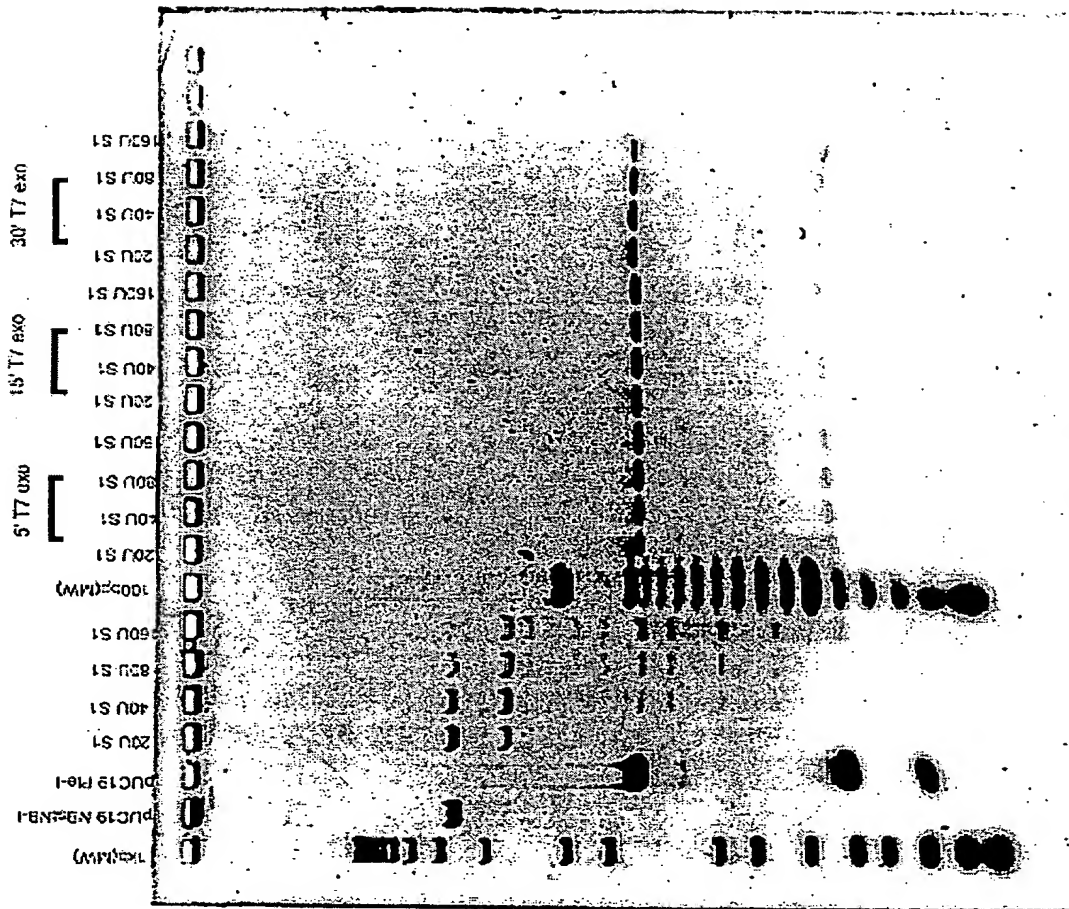


FIG. 75

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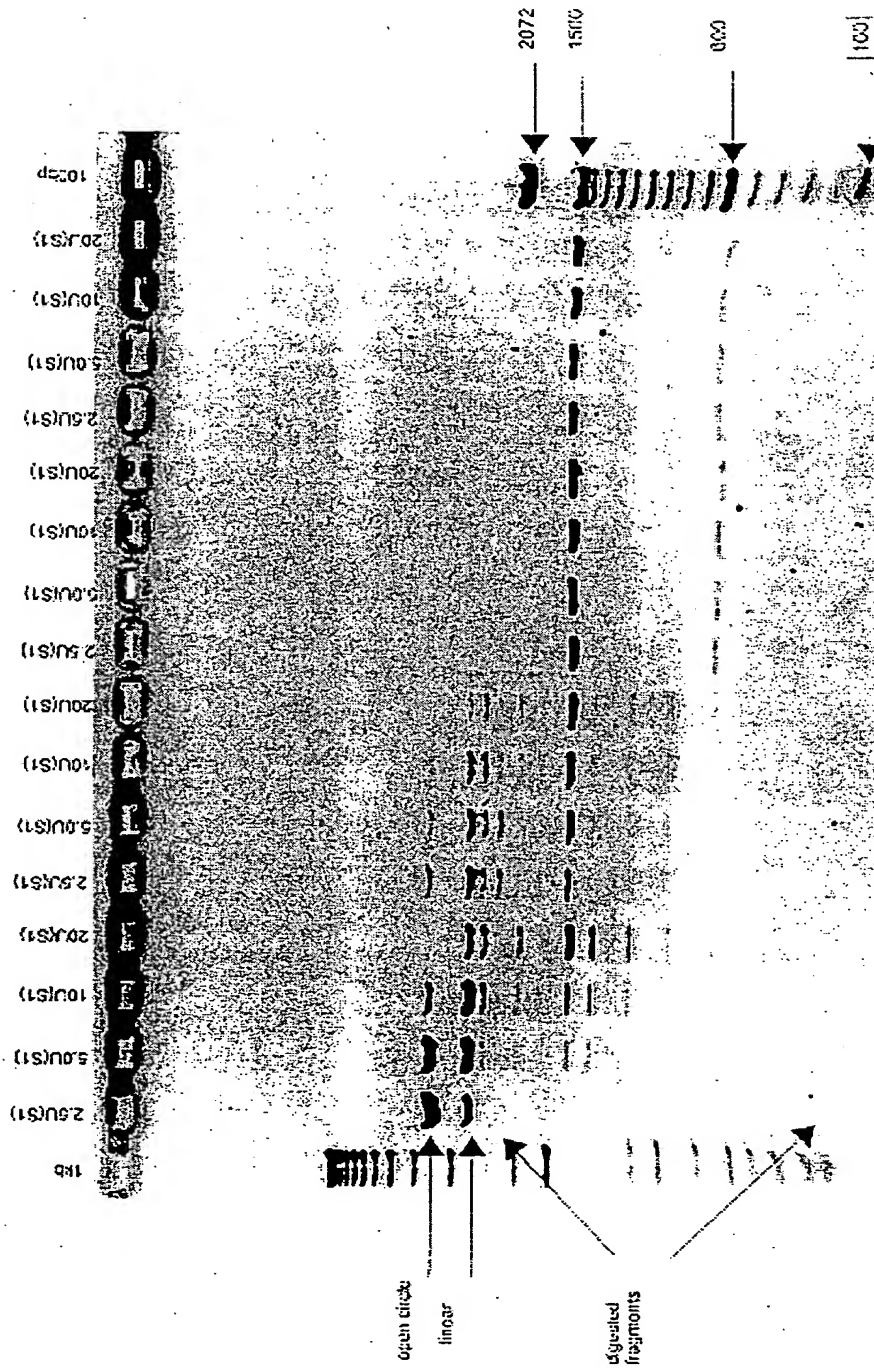


FIG. 76



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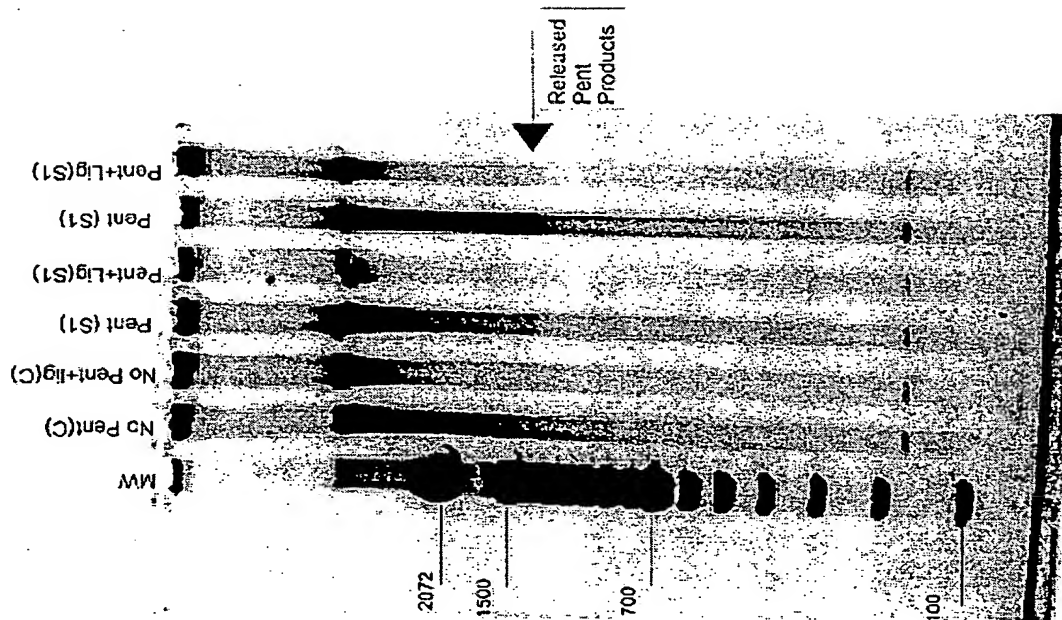
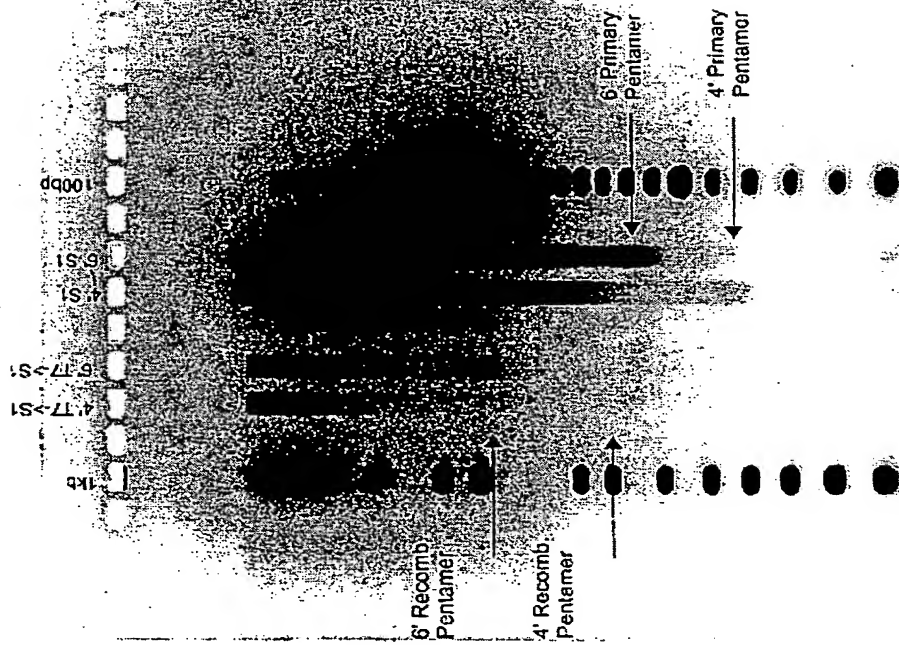
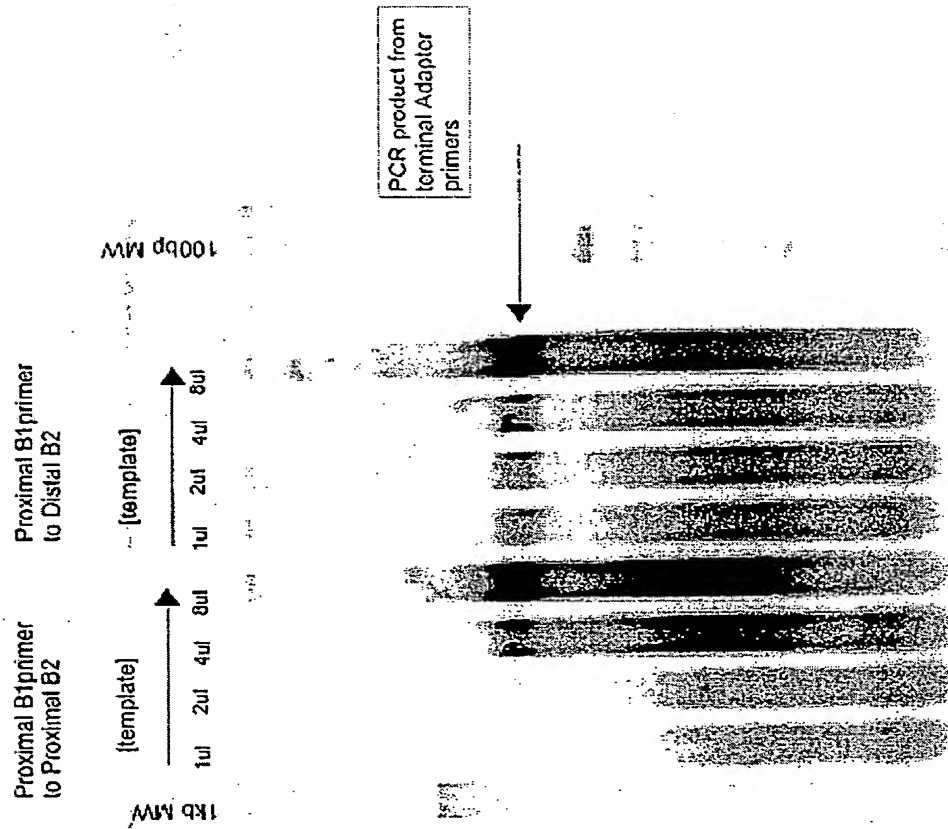


FIG. 77



Enzymatic Release of Recombinant  
Pentamers T7 gene6 - S1 nuclease

FIG. 78



Amplification of Secondary Nick Translation released recombinant Pentamers

FIG. 79

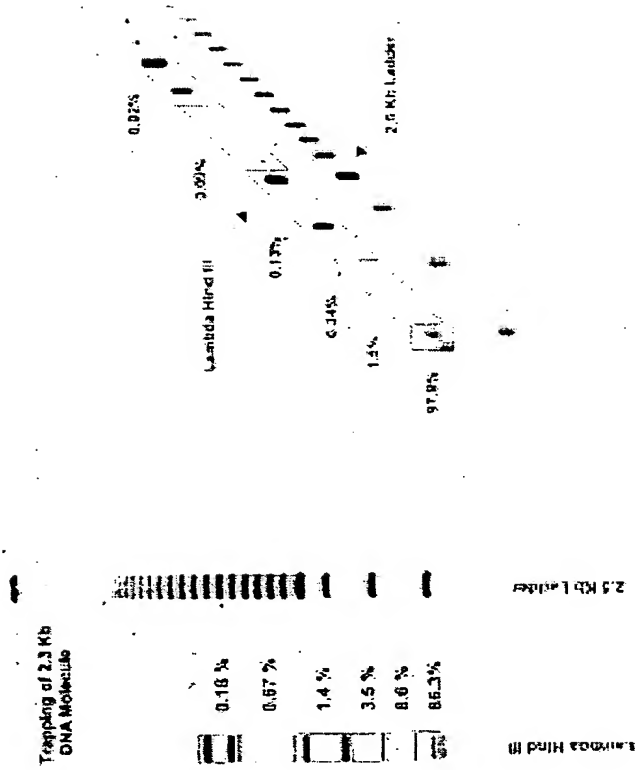


FIG. 80A

FIG. 80B

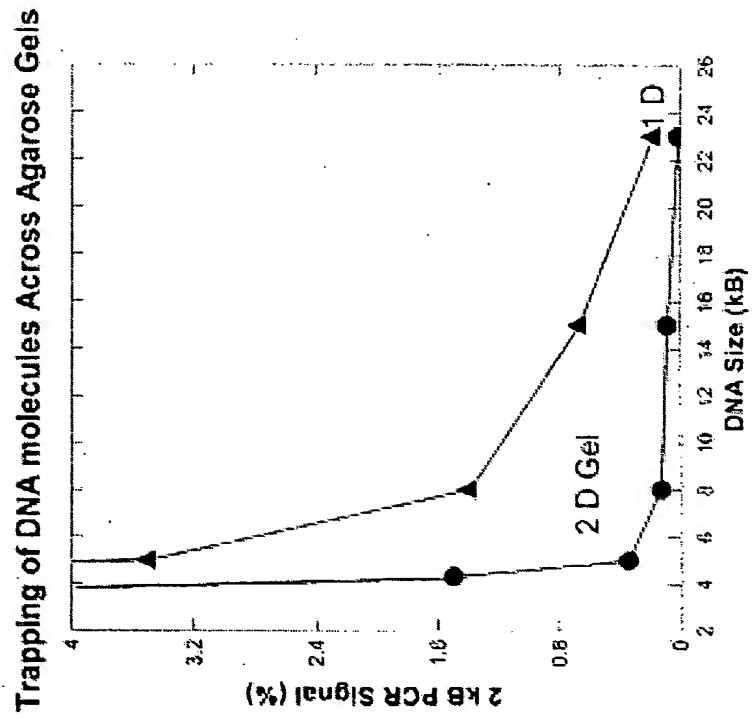


FIG. 81

# Recovery of DNA Fragments after Microcon YM-100 Filtration

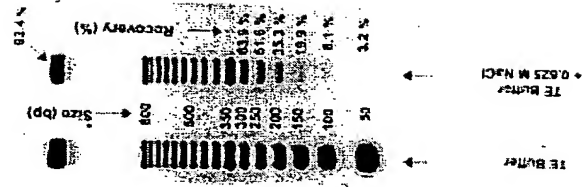


FIG. 82

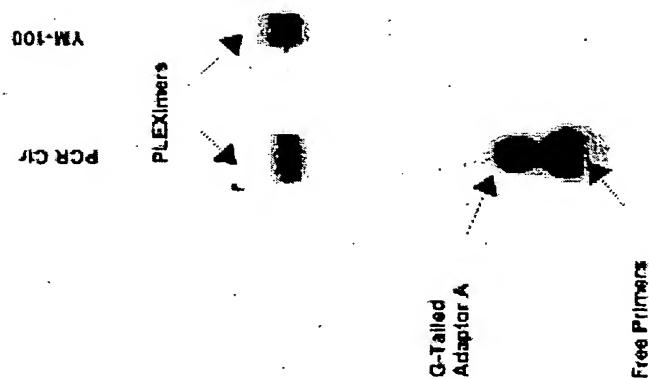


FIG. 83

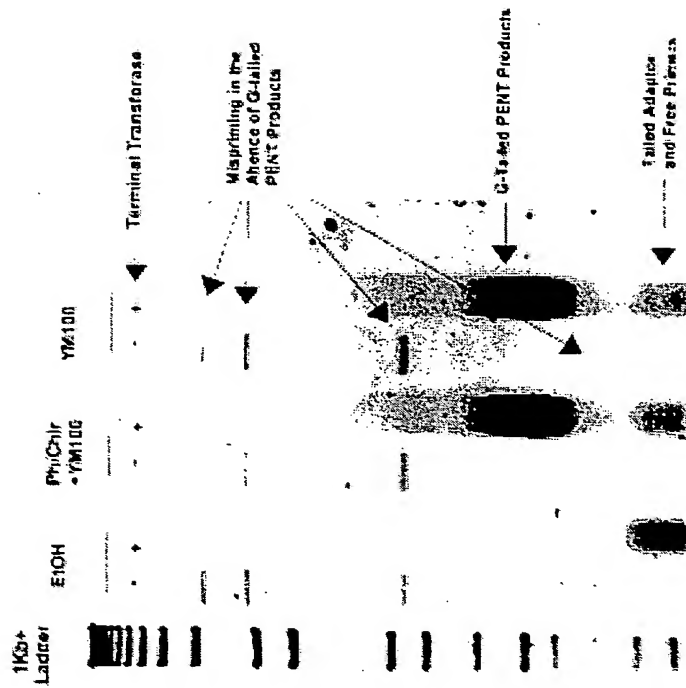


FIG. 84



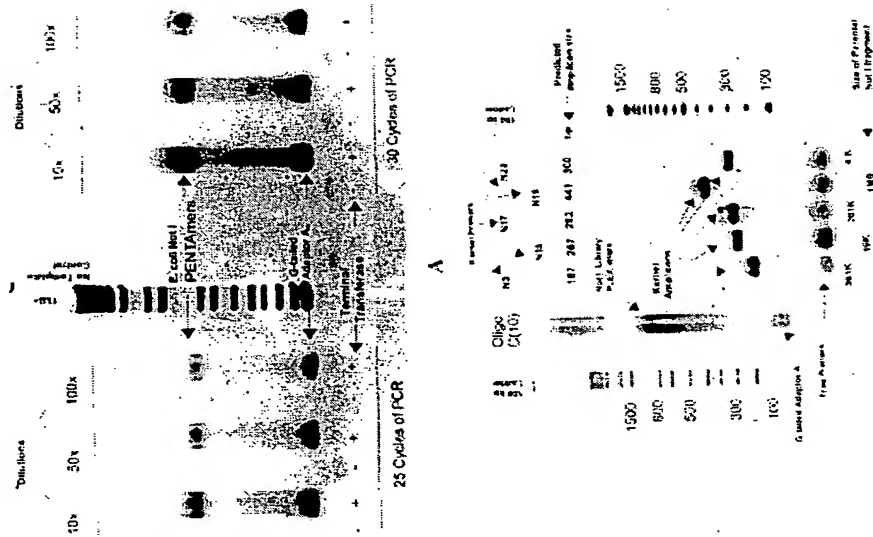
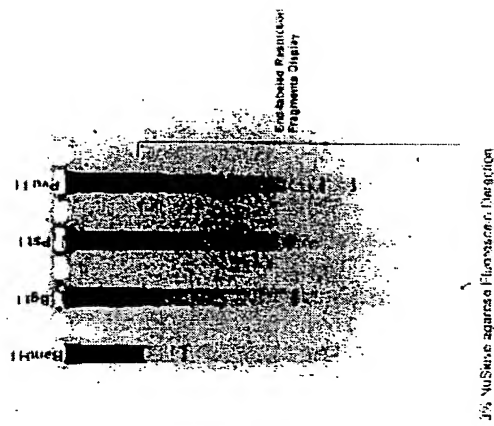


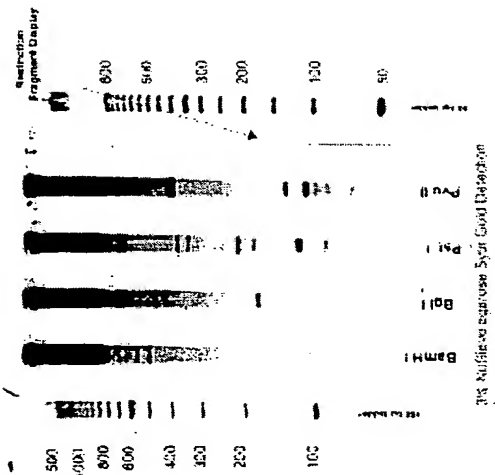
FIG. 85



FIG. 86



A



B

FIG. 87

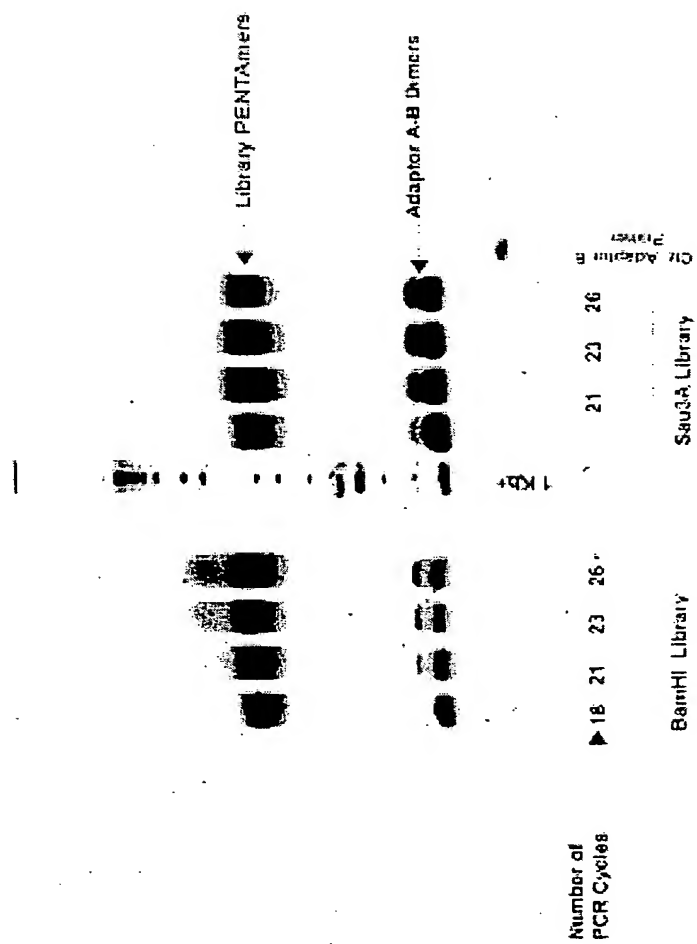


FIG. 88

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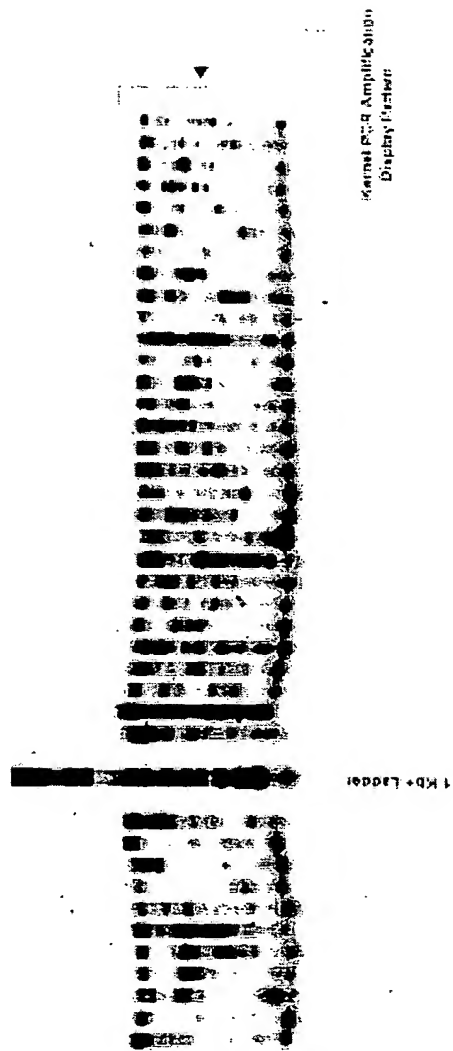


FIG. 89

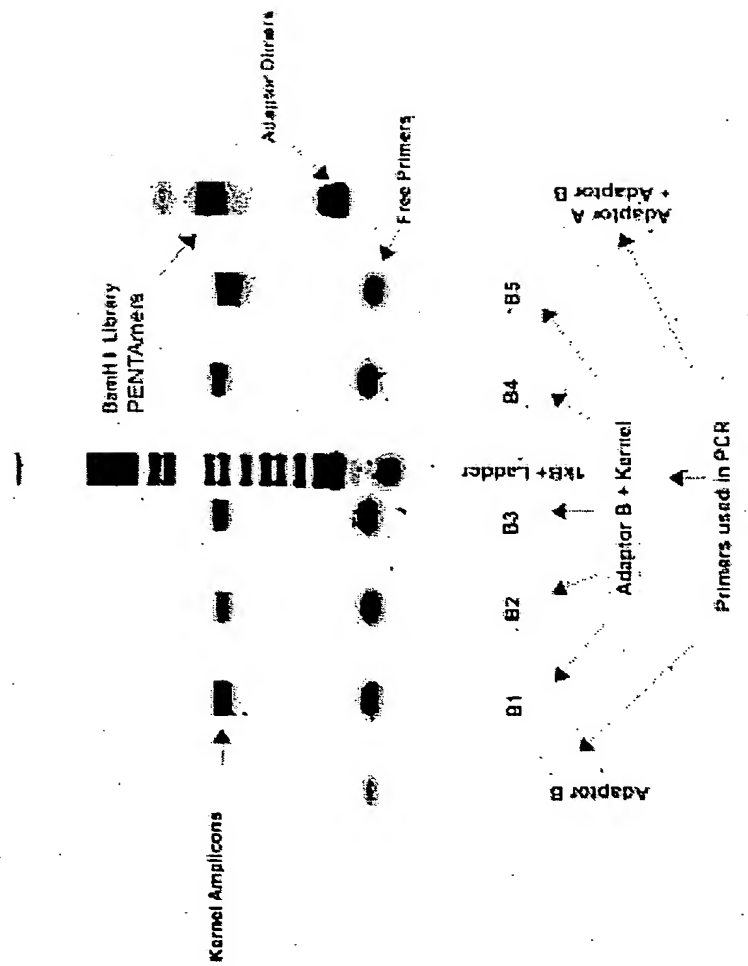


FIG. 90

